Chapter 6
Nitric Oxide Contributes to Retinal Ganglion Cell Survival Through Protein S-Nitrosylation After Optic Nerve Injury

Yoshiki Koriyama and Satoru Kato

Abstract Neuroprotective strategies to attenuate retinal ganglion cell (RGC) death could lead to novel therapies for chronic optic neuropathies such as glaucoma. Nitric oxide (NO) signaling results in both neurotoxic and neuroprotective effects in CNS neurons after nerve lesion. However, the functional mechanisms of NO in the nervous system are not fully understood. Protein S-nitrosylation by NO is a posttranslational modification that regulates protein function through the reaction of NO with a cysteine thiol group on target proteins. NO/S-nitrosylation is now thought to be important in regulating cell death, survival, and gene expression. However, there are few reports on the role of protein S-nitrosylation in glaucoma. Therefore, we investigated the role of protein S-nitrosylation signaling in RGC survival after optic nerve injury.

Keywords Glaucoma • Keap1 • Nitric oxide • Nrf2 • Retinal ganglion cell • S-nitrosylation

6.1 NO Signaling in Glaucoma

6.1.1 Nerve Injury Model of Glaucoma in Rodents

Glaucoma is a neurodegenerative disorder characterized by the progressive loss of retinal ganglion cells (RGCs) and by degeneration of optic axons. Elevated intraocular pressure is considered to be one of the major risk factors associated with this

Y. Koriyama (✉) • S. Kato
Department of Molecular Neurobiology, Graduate School of Medicine, Kanazawa University, 13-1 Takaramachi, Kanazawa 920-8640, Japan

Graduate School and Faculty of Pharmaceutical Sciences, Suzuka University of Medical Sciences, 3500-3 Minamitamagaki, Suzuka 513-8670, Japan
e-mail: koriyama@suzuka-u.ac.jp

T. Nakazawa et al. (eds.), *Neuroprotection and Neuroregeneration for Retinal Diseases*, DOI 10.1007/978-4-431-54965-9_6, © Springer Japan 2014

neuropathy [1]. However, in some glaucomatous patients, loss of RGCs and a subsequent loss of vision can occur even with normal intraocular pressure. Although the causes of glaucoma are unclear, the various pathogenetic mechanisms of glaucoma result in the common end stage of RGC apoptosis. For example, almost 90 % of RGCs die within 2 weeks after optic nerve injury [2]. In particular, oxidative stress and nitrative stress appear to play important roles in this progressive neuronal death. Eye tissue uses four times more oxygen than brain tissue and thus is highly exposed to various reactive oxygen species (ROS) including hydrogen peroxide, hydroxyl radicals, and superoxide anions. Consequently, eye tissue contains very high amounts of antioxidants, such as superoxide dismutase, catalase, ascorbate, and vitamins [3]. In the pathogenetic stages of RGC death, increasing intracellular ROS generation has been reported to accompany glutathione depletion [4]. Evidence also indicates that there are reductions in endogenous antioxidants in aging and induction of lipid peroxidation [5]. On the other hand, mechanical injury of axons, and thereby a lack of neurotrophins being supplied to RGC bodies, is one of the mechanisms proposed for the retrograde degeneration of RGCs following optic nerve injury [2]. RGC death after nerve injury is mostly caused by apoptosis associated with upregulation of proapoptotic Bax and downregulation of antiapoptotic Bcl-2 and Bcl-xL [6]. As RGC cell loss and optic nerve degeneration in the crush injury model mimics many of the features of glaucoma, we use this model to study the mechanisms of RGC survival and/or axonal regeneration [7].

Nitric oxide (NO) levels also increase in many retinal cells within a few days of optic nerve damage [8]. Many reports suggest that excess NO plays a crucial role in neuronal cell death. However, NO can also prevent neuronal cell death. In general, NO mediates neuroprotection through two main signaling pathways: the NO/cyclic guanosine monophosphate (cGMP) pathway and the protein S-nitrosylation pathway. However, whether S-nitrosylation of target protein promotes RGC survival after injury is unknown. This is the focus of this review.

6.1.2 Role of Three Isoforms of Nitric Oxide Synthase in Glaucomatous Retina

NO is an important signaling molecule that regulates a range of physiological processes, including vasodilatation, neuronal function, inflammation, and immune function [9]. NO is an organic gas ubiquitously synthesized by NO synthase (NOS). In mammalian cells, NOS is subclassified into three types: brain or neuronal NOS (nNOS or NOSI), inducible NOS (iNOS or NOSII), and endothelial NOS (eNOS or NOSIII). The function of NO is different depending on the cell type and enzyme isotype.

nNOS may be involved in neurotransmission by creating retrograde signaling between synapses. At synapses, nNOS is coupled to *N*-methyl-D-aspartate receptors (NMDA-R) via postsynaptic density-95 protein complexes [10]. Upon glutamate stimulation of NMDA-R, calcium ions enter the cytoplasm through the ion channel.

In conjunction with calmodulin, calcium ion influx triggers nNOS activation and NO generation [11]. Low levels of NO (picomole order) that are produced under physiological conditions stimulate many normal intracellular signaling pathways. In contrast, overstimulation of NMDA-R and subsequent calcium ion influx promote pathological signaling, resulting in neural damage and death through production of toxic amounts of NO [12]. Increased expression of nNOS in the retina and optic nerve head was reported in rats with elevated intraocular pressure. Most axotomized RGCs express nNOS protein, and these cells degenerate within 2 weeks after optic nerve injury [13]. Excessive NO generated from injured RGCs might be one risk factor for RGC cell death in glaucoma.

iNOS can be upregulated by acute inflammatory stimuli. For example, neurotoxic levels of NO (nanomole order [14]) via iNOS induction in activated glial cells give arise various neurodegenerative diseases. NO will further oxidize to nitrite, peroxynitrite, and free radicals to highly interact with thiols and iron–sulfur centers of various enzymes [14] to alter the biological function of cells and result in apoptosis, neurotoxicity, optic nerve degeneration, and numerous eye diseases. It has been reported that glaucoma could be due to neurotoxic effects of NO at the optic nerve head and in the RGCs which results in optic nerve head degeneration and visual field loss [15]. Inducible NOS was triggered by ocular inflammation, LPS, endotoxin, or cytokines including tumor necrosis factor α (TNFα) and interleukin-1 or interleukin-6 [16]. Therefore, one possibility for the treatment of glaucoma could be the use of inhibitors of iNOS induction and/or its activity. In the retina, Müller glial cells can express the iNOS isoform after endotoxin and cytokine exposure [17]. Retinal pigment epithelium (RPE) cells also contain iNOS in various species [18]. Goureau et al. [19] reported that fibroblast growth factors (FGFs) and transforming growth factor β (TGFβ) have opposite effects on the regulation of the production of NO in RPE cells. FGFs inhibit the induction of iNOS at the transcriptional level. Conversely, TGFβ frequently acts as an immunosuppressor. TGFβ attenuates NO production in human and rat RPE and Müller glial cells [20]. These molecules might be candidates to reverse or treat NO-related glaucoma. On the other hand, upregulation of TNFα and TNFα receptor-1 expression was accompanied by progressive optic nerve degeneration in the glaucomatous optic nerve head [21]. It has been reported that TNFα contributes to the progression of optic nerve degeneration by inducing iNOS expression in glial cells. Thus, the TNFα inhibitor etanercept or other antagonists of TNFα or suppressors of inflammation could be considered as therapeutic tools against glaucoma [22].

The major function of eNOS is vasodilation, by regulating vascular smooth muscle relaxation. Immunoreactivity of eNOS is seen in the retinal vascular endothelial cells, choroid and retina [23]. In glaucomatous eyes, overexpression of nNOS and iNOS is linked to glaucomatous RGC apoptosis through increased levels of NO, while enhanced staining for eNOS is assumed to be a compensatory neuroprotective reaction [24]. Furthermore, no significant changes in eNOS expression have been observed in the chronic glaucoma model.

The reagents that can regulate NO levels in the retina could become a reasonable neuroprotective agent for treating glaucoma.

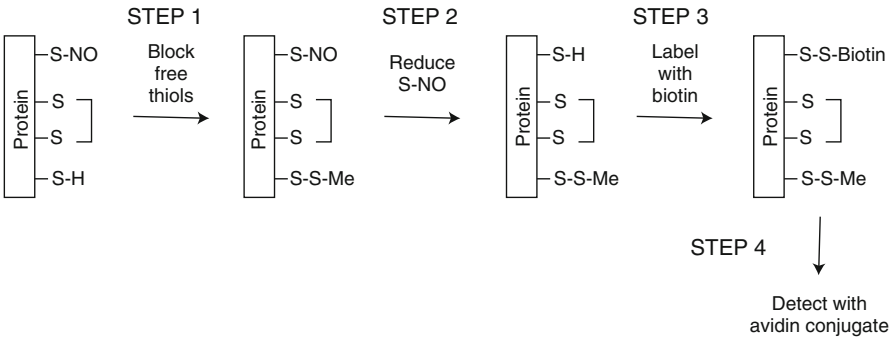

Fig. 6.1 Schematic diagram for detecting S-nitrosylated proteins (biotin-switch assay). Step 1: free thiols are blocked by methylthiolation reagent. Step 2: the reduction of SNO bonds to thiols with reductant. Step 3: newly reformed cysteines react with biotin-conjugated thiol-modifying reagent. Step 4: target biotinylated proteins are collected by avidin-coupled reagents

6.1.3 Anti- and Proapoptotic Mechanisms by NO and Protein S-Nitrosylation

Because NO is a free gas, it can easily penetrate the biological membrane. Superoxide radical shows a high affinity toward NO. The reaction between NO and superoxide anion produces peroxynitrite [25], a highly reactive molecule that can cause extensive damage to proteins, lipids, and DNA molecules. Furthermore, NO can react with thiols, organosulfur compounds containing a carbon-bonded sulfhydryl (–C–SH or R–SH) group. Some actions of NO, including neurotransmission and vasodilation, are mediated via the activation of soluble guanylate cyclase and subsequent elevation of cGMP levels [26]. Other actions of NO are mediated via S-nitrosylation of the free cysteine SH group of proteins and regulate their activities when cysteines are present at their active site [26].

Since Jafferey and Snyder discovered the "biotin-switch assay" for protein S-nitrosylation [27], nearly 1,000 proteins have been identified as *S*-nitrosoproteins. The biotin-switch assay consists of four steps (Fig. 6.1):

- Step 1—the methylthiolation of free cysteine thiols with methyl methanethiosulfonate.
- Step 2—the reduction of SNO bounded to thiols with reductant.
- Step 3—newly reformed cysteines are reacted with biotin-conjugated thiol-modifying reagent.
- Step 4—target biotinylated proteins are collected by avidin-coupled reagents.

S-nitrosylation can mediate either neuroprotective or neurotoxic effects, depending on the action of the target protein [28]. For the neuroprotective effect, S-nitrosylation of caspases inhibits their activation. Most caspases contain a single cysteine at their catalytic site, which is susceptible to redox modification and can be effectively modified by S-nitrosylation in the presence of NO with subsequent

Table 6.1 S-nitrosylated proteins targeting for cell survival/death

Functions	Name	References
Cell survival/death	14-3-3	[41]
	Apoptosis signal-regulating kinase 1	[42]
	Bcl-2	[43]
	Caspases	[30]
	Cyclin-dependent kinase 5	[44]
	Cyclooxygenase-2	[45]
	Dynamin-related protein 1	[46]
	Erk	[47]
	Fas	[48]
	GAPDH	[34]
	GOSPEL	[49]
	Keap1	[50, 51]
	Matrix metalloproteinase 9	[52]
	Parkin	[37]
	Peroxiredoxin2	[53]
	PTEN	[39, 40]
	STAT3	[54]
	X-linked inhibitor of apoptosis protein	[55]

inhibition of enzyme activity [29]. S-nitrosylation has been shown to reduce the activity of caspases such as caspase-3, caspase-8, and caspase-9 in various types of neurons [30]. These results indicate that endogenous NO generated by NOS exerts an antiapoptotic function by S-nitrosylation-dependent inactivation of caspases. In contrast, another target protein of S-nitrosylation promotes activation of caspases and induces cell death. Caspase activation during NO stimulation also occurs as a result of downregulation of X-linked inhibitor of apoptosis protein (XIAP) [31]. Under normal conditions, XIAP efficiently binds to the catalytic sites of caspases and inhibits them [32]. However, NO inactivates the E3 ligase activity of XIAP through S-nitrosylation, thus stabilizing caspases [33]. On the other hand, various S-nitrosylated target proteins are also involved in cell death signaling. For example, NO S-nitrosylates glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and binds to Siah, an E3 ubiquitin ligase [34]. Inside the nucleus, S-nitrosylated GAPDH stabilizes Siah, and the complex facilitates ubiquitination and degradation of the nuclear coreceptor [35]. This mechanism is thought to regulate gene expression associated with cellular dysfunction and death [36]. Recent studies have shown unregulated S-nitrosylation of many proteins involved in neuronal death and neurodegenerative disorders, such as Parkin [37], protein disulfide-isomerase (PDI) [38], and phosphatase and tensin homologue deleted on chromosome 10 (PTEN) [39, 40]. The target S-nitrosylated proteins for cell death or cell survival are listed in Table 6.1. In the future, there might be new targets that could be used for the treatment of glaucoma.

6.2 Antioxidative Effects of NO Through Keap1 S-Nitrosylation Signaling in RGCs

6.2.1 Protective Effects of Nip Against Oxidative Stress

Although a growing number of S-nitrosylated proteins are reported to contribute to pathogenetic brain disease, there are few such reports for retinal diseases. Therefore, we studied the role of protein S-nitrosylation by NO in RGC survival and axonal regeneration after optic nerve injury [50, 51, 56–58]. Many chemicals such as glyceryl trinitrate, sodium nitroprusside, and S-nitrosothiols have been reported as NO donors [59]. Recently, novel hybrid NO donor drugs have been designed as NO-releasing compounds such as NO–NSAIDS and nipradilol (Nip), which already has been registered as an antiglaucoma agent in Japan. Nip acts as a vasodilator by releasing NO from the nitroxy moiety [60]. Nip lowers intraocular pressure via both selective α1-adrenoceptor and nonselective β-adrenoceptor antagonists. The protective effects of Nip have been shown in various neuronal cells [61] including RGCs [62]. Mizuno et al. demonstrated that the protective effects of Nip were NO dependent because both selective α1- and nonselective β-adrenoceptor antagonists had no effect on RGCs [63]. However, the detailed mechanism of NO-dependent protection is not clear. Several lines of evidence indicate that NO can suppress RGC cell death [64] through an NO/cGMP-dependent pathway [65]. Furthermore, Tomita et al. [66] reported that the beneficial effect of Nip on RGC cell death was partially cancelled by inhibiting protein kinase G. Moreover, Naito [67] reported that Nip attenuates hydrogen peroxide-induced lipid peroxidation. Thus, we wanted to know whether the neuroprotective action of Nip is mediated by antioxidative processes via a NO/cGMP- or a NO/S-nitrosylation-dependent mechanism. In addition, as Nip did not S-nitrosylate caspase-3 under our experimental conditions, we focused on the possibility of Kelch-like ECH-associated protein 1 (Keap1) S-nitrosylation to understand its antioxidative action.

The Keap1 and NF-E2-related factor 2 (Nrf2) pathways regulate the expression of cytoprotective genes in response to oxidative stress or electrophilic stress [68]. Keap1 is the redox-sensor protein that allows the activation of Nrf2 by modification including oxidation or S-nitrosylation [69]. Once activated, Nrf2 translocates from the cytosol to the nucleus, binds to the antioxidant responsible element (ARE) of target genes, and drives their expression of antioxidative heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase-1 (NQO-1), and glutamate cysteine ligase C (GCLC). Keap1 sensor protein contains 27 cysteine residues. Several analyses have identified multiple Keap1 cysteine residues to be involved in the reaction with oxygen or electrophiles. The most frequently reported targets are Cys151, Cys257, Cys273, Cys288, Cys297, and Cys613 [70, 71]. However, there are notable differences between laboratories, electrophilic probes, and species. Interestingly, NO activates the Keap1/Nrf2 pathway by S-nitrosylation of Keap1 protein in colon carcinoma cells [72]. Two reactive cysteines, Csy273 and Cys288, of Keap1 have been identified as key sites of translocational activity of Nrf2 [73].

Thus, we focused on the neuroprotective mechanism of Nip thorough Keap1 S-nitrosylation.

We first tested whether Nip could induce NO generation in a retinal ganglion cell line: RGC-5 [51]. Nip (20 μM) significantly increased (1.6-fold) fluorescence intensity of NO indicator compared to no treatment (control) within 1 h of treatment. Denitro-nipradilol (DeNip) has weak selective α1-adrenoceptor and nonselective β-adrenoceptor antagonist properties, but no NO-donating action. DeNip did not increase NO production in RGC-5 cells.

To evaluate the protective action of Nip against oxidative stress in RGC-5 cells, we used oxidative stress models in culture with hydrogen peroxide, tBOOH, and serum withdrawal. Charles et al. [74] studied the signaling cascades involved in RGC-5 cell death under serum deprivation. They demonstrated that serum deprivation increases malondialdehyde and decreases reduced glutathione. Furthermore, several groups have already reported that serum deprivation produced ROS in RGC-5 cells [75]. While pretreatment with Nip 4–6 h before oxidative stress exposure showed the maximum protective effect, no protective effect was observed when Nip was applied to RGC-5 0–2 h before oxidative stress stimulation. Another NO donor, NOR1, also showed protective effects against oxidative stress [50]. These protective effects were reversed by a NO scavenger. The protective effects of Nip were suppressed by the protein synthesis inhibitor cycloheximide. In contrast, DeNip did not show any neuroprotective effect against oxidative stress.

6.2.2 NO/S-Nitrosylation-Dependent Antioxidative Protein Induction by Nip

As the protective effect of Nip was dependent upon newly synthesized proteins, we tested the inducibility of antioxidative enzymes in RGC-5 cells. After 4 h of Nip treatment, we found that heme oxygenase-1 (HO-1) mRNA and protein had increased in RGC-5 cells. HO-1 [76] is an enzyme that degrades intracellular heme to free iron, carbon monoxide, and biliverdin. Bilirubin, converted from biliverdin, acts as a strong endogenous ROS scavenger and attenuates lipid peroxidation related to 4-hydroxy-2-nonenal (4HNE). Other antioxidative proteins, such as NQO-1 and GCLC, did not increase for up to 12 h after treatment of Nip. DeNip did not alter HO-1 expression. These results indicate that the protective effect of Nip is dependent on NO. An HO-1 inhibitor prevented the neuroprotective effect of Nip against oxidative stress. The data indicate that the neuroprotective action of Nip is caused by NO generation following antioxidative HO-1 expression.

To test the involvement of the Keap1/Nrf2 system in the Nip-induced increase in HO-1 expression, we analyzed the translocation of Nrf2 to the nucleus. Nip facilitated the translocation of Nrf2 into the nucleus. Both a NO scavenger and an S-nitrosylation blocker (dithiothreitol) inhibited nuclear translocation of Nrf2 by Nip. Furthermore, translocated Nrf2 was bound to the E1 enhancer of HO-1 promoter as an ARE site [50]. These results suggest that Nip-mediated translocation

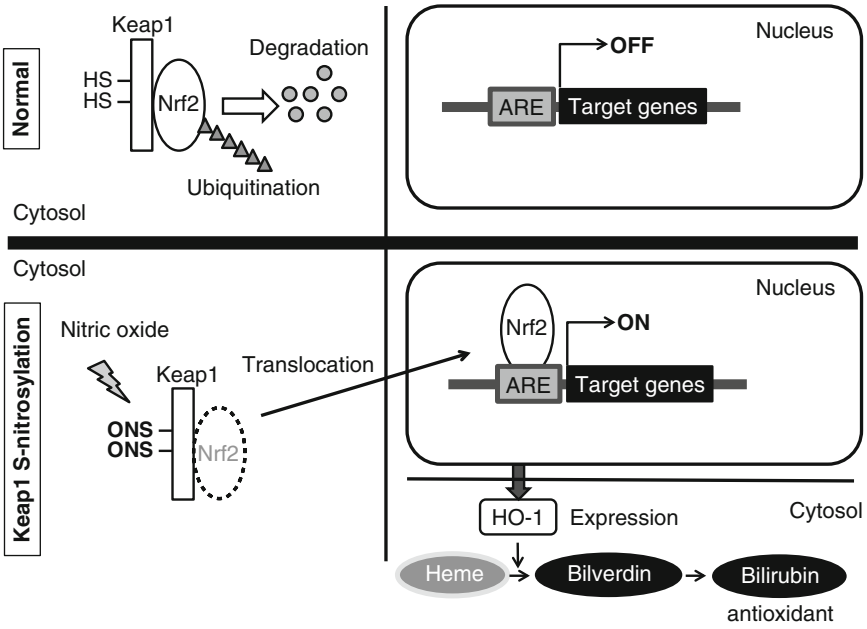

Fig. 6.2 Schematic diagram of Keap1 S-nitrosylation-dependent antioxidative signaling pathway. NO donors induce S-nitrosylation of Keap1 and thereafter HO-1 expression through Nrf2/ARE signaling

of Nrf2 to the nucleus is dependent on NO/S-nitrosylation pathways. We further investigated the effects of the NO scavenger and S-nitrosylation blocker on HO-1 induction by Nip. As expected, the increase in HO-1 was blocked by both reagents. By using the biotin-switch assay, we determined that Nip increased S-nitrosylated Keap1 but not caspase-3.

To extend the protective effect of Nip in vivo, we used an optic nerve injury model in mice. Nip suppressed the final products of lipid peroxidation: 4HNE accumulation mediated by inducing HO-1 expression in RGCs after nerve injury. Finally, RGC death after nerve injury was reduced by Nip. These results demonstrate for the first time that Nip protects RGC death against oxidative stress both in vitro and in vivo through the induction of HO-1 by S-nitrosylation of Keap1 (Fig. 6.2). This novel neuroprotective action of Nip in RGCs may shed additional light on possible antiglaucomatous agents.

6.3 PTEN S-Nitrosylation-Induced Optic Nerve Regeneration by Nip

Nip has also been reported as having neuritogenic action in cat RGCs [77]. However, the mechanism of Nip-induced optic nerve regeneration has not been fully elucidated. It has been reported that PTEN deletion strongly showed optic nerve

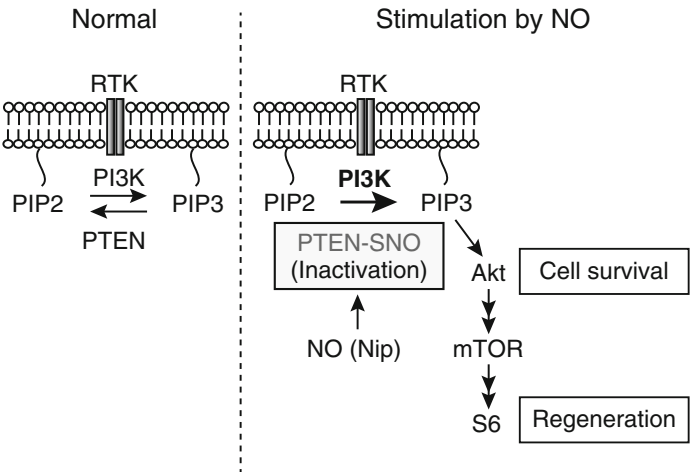

Fig. 6.3 Schematic diagram of PTEN S-nitrosylation-dependent axonal regeneration. In the case of NO stimulation, PTEN was inactivated through S-nitrosylation. Subsequently, Akt phosphorylates a wide range of substrates involved in the regulation of cellular functions, including cell growth and survival. Akt activation also induced mTOR/S6 signaling, which is known as a novel pathway of axonal regeneration. *RTK*, tyrosine kinase receptor; *PIP2*, phosphatidylinositol (4, 5) biphosphate; *PIP3*, phosphatidylinositol (3, 4, 5) triphosphate

regeneration after injury in part by increasing protein translation through the mammalian target of rapamycin (mTOR) pathway [78–81]. PTEN and mTOR are critical factors for controlling the regenerative capacity of mouse RGCs and corticospinal neurons [81]. It has been known that PTEN is inactivated by S-nitrosylation and then activates phosphoinositide 3-kinase (PI3K) and its downstream pathway [39]. We showed a correlation between Akt/mTOR activities and optic nerve regeneration through S-nitrosylation of PTEN in RGCs [80] (Fig. 6.3).

6.4 Future Studies

For treatment of glaucomatous degeneration, neuroprotective and neuritogenic actions in RGCs play a central role. As there are a few reports on protein S-nitrosylation in glaucoma, elucidating specific targets of S-nitrosylation and understanding their regulatory mechanism could assist the development of therapeutic intervention and be a next-era target for the treatment of injured RGCs in glaucomatous retina.

References

1. García-Campos J, Villena A, Díaz F et al (2007) Morphological and functional changes in experimental ocular hypertension and role of neuroprotective drugs. Histol Histopathol 22:1399–1411
2. Berkelaar M, Clarke DB, Wang YC et al (1994) Axotomy results in delayed death and apoptosis of retinal ganglion cells in adult rats. J Neurosci 14:4368–4374
3. Anderson B Jr, Saltzman HA (1964) Retinal oxygen utilization measured by hyperbaric blackout. Arch Ophthalmol 72:792–795
4. Maher P, Hanneken A (2005) Flavonoids protect retinal ganglion cells from oxidative stress-induced death. Invest Ophthalmol Vis Sci 46:4796–4803
5. Wang AL, Lukas TJ, Yuan M et al (2010) Age-related increase in mitochondrial DNA damage and loss of DNA repair capacity in the neural retina. Neurobiol Aging 31:2002–2010
6. Chierzi S, Strettoi E, Cenni MC et al (1999) Optic nerve crush: axonal responses in wild-type and bcl-2 transgenic mice. J Neurosci 19:8367–8376
7. Levkovitch-Verbin H, Harris-Cerruti C, Groner Y et al (2000) RGC death in mice after optic nerve crush injury: oxidative stress and neuroprotection. Invest Ophthalmol Vis Sci 41:4169–4174
8. Groppe M, Thanos S, Schuhmann W et al (2003) Measurement of nitric oxide production by the lesioned rat retina with a sensitive nitric oxide electrode. Anal Bioanal Chem 376:797–807
9. Calabrese V, Cornelius C, Rizzarelli E et al (2009) Nitric oxide in cell survival: a janus molecule. Antioxid Redox Signal 11:2717–2739
10. Sattler R, Xiong Z, Lu WY et al (1999) Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. Science 284:1845–1848
11. Bredt DS, Hwang PM, Glatt CE et al (1991) Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. Nature 351:714–718
12. Lipton SA, Choi YB, Pan ZH et al (1993) A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature 364:626–632
13. Lee EJ, Kim KY, Gu TH et al (2003) Neuronal nitric oxide synthase is expressed in the axotomized ganglion cells of the rat retina. Brain Res 986:174–180
14. Chiou GC (2001) Review: effects of nitric oxide on eye diseases and their treatment. J Ocul Pharmacol Ther 17:189–198
15. Neufeld AH, Sawada A, Becker B (1999) Inhibition of nitric-oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma. Proc Natl Acad Sci USA 96:9944–9948
16. Dawson VL, Dawson TM (1995) Physiological and toxicological actions of nitric oxide in the central nervous system. Adv Pharmacol 34:323–342
17. Goureau O, Hicks D, Courtois Y et al (1994) Induction and regulation of nitric oxide synthase in retinal Müller glial cells. J Neurochem 63:310–317
18. Liversidge J, Grabowski P, Ralston S et al (1994) Rat retinal pigment epithelial cells express an inducible form of nitric oxide synthase and produce nitric oxide in response to inflammatory cytokines and activated T cells. Immunology 83:404–409
19. Goureau O, Lepoivre M, Becquet F et al (1993) Differential regulation of inducible nitric oxide synthase by fibroblast growth factors and transforming growth factor beta in bovine retinal pigmented epithelial cells: inverse correlation with cellular proliferation. Proc Natl Acad Sci USA 90:4276–4280
20. Becquet F, Courtois Y, Goureau O (1997) Nitric oxide in the eye: multifaceted roles and diverse outcomes. Surv Ophthalmol 42:71–82
21. Yuan L, Neufeld AH (2000) Tumor necrosis factor-alpha: a potentially neurodestructive cytokine produced by glia in the human glaucomatous optic nerve head. Glia 32:42–50

22. Roh M, Zhang Y, Murakami Y et al (2012) Etanercept, a widely used inhibitor of tumor necrosis factor-α (TNF-α), prevents retinal ganglion cell loss in a rat model of glaucoma. PLoS One 7:e40065
23. Chakravarthy U, Stitt AW, McNally J et al (1995) Nitric oxide synthase activity and expression in retinal capillary endothelial cells and pericytes. Curr Eye Res 14:285–294
24. Neufeld AH (1999) Nitric oxide: a potential mediator of retinal ganglion cell damage in glaucoma. Surv Ophthalmol 43:129–135
25. Beckman JS, Beckman TW, Chen J et al (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 87:1620–1624
26. Schmidt HH, Lohmann SM, Walter U (1993) The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. Biochim Biophys Acta 1178:153–175
27. Jaffrey SR, Erdjument-Bromage H, Ferris CD et al (2001) Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. Nat Cell Biol 3:193–197
28. Nakamura T, Lipton SA (2011) Redox modulation by S-nitrosylation contributes to protein misfolding, mitochondrial dynamics, and neuronal synaptic damage in neurodegenerative diseases. Cell Death Differ 18:1478–1486
29. Melino G, Bernassola F, Knight RA et al (1997) S-nitrosylation regulates apoptosis. Nature 388:432–433
30. Tenneti L, D'Emilia DM, Lipton SA (1997) Suppression of neuronal apoptosis by S-nitrosylation of caspases. Neurosci Lett 236:139–142
31. Manderscheid M, Messmer UK, Franzen R et al (2001) Regulation of inhibitor of apoptosis expression by nitric oxide and cytokines: relation to apoptosis induction in rat mesangial cells and raw 264.7 macrophages. J Am Soc Nephrol 12:1151–1163
32. Yang YL, Li XM (2000) The IAP family: endogenous caspase inhibitors with multiple biological activities. Cell Res 10:169–177
33. Nakamura T, Wang L, Wong CC et al (2010) Transnitrosylation of XIAP regulates caspase-dependent neuronal cell death. Mol Cell 39:184–195
34. Hara MR, Agrawal N, Kim SF et al (2005) S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. Nat Cell Biol 7:665–674
35. Zhang J, Guenther MG, Carthew RW et al (1998) Proteasomal regulation of nuclear receptor corepressor-mediated repression. Genes Dev 12:1775–1780
36. Sen N, Hara MR, Kornberg MD et al (2008) Nitric oxide-induced nuclear GAPDH activates p300/CBP and mediates apoptosis. Nat Cell Biol 10:866–873
37. Sunico CR, Nakamura T, Rockenstein E et al (2013) S-nitrosylation of parkin as a novel regulator of p53-mediated neuronal cell death in sporadic Parkinson's disease. Mol Neurodegener 8:29
38. Walker AK, Farg MA, Bye CR et al (2010) Protein disulphide isomerase protects against protein aggregation and is S-nitrosylated in amyotrophic lateral sclerosis. Brain 133:105–116
39. Kwak YD, Ma T, Diao S et al (2010) NO signaling and S-nitrosylation regulate PTEN inhibition in neurodegeneration. Mol Neurodegener 5:49
40. Numajiri N, Takasawa K, Nishiya T et al (2011) On-off system for PI3-kinase-Akt signaling through S-nitrosylation of phosphatase with sequence homology to tensin (PTEN). Proc Natl Acad Sci USA 108:10349–10354
41. Kuncewicz T, Sheta EA, Goldknopf IL et al (2003) Proteomic analysis of S-nitrosylated proteins in mesangial cells. Mol Cell Proteomics 2:156–163
42. Sumbayev VV (2003) S-nitrosylation of thioredoxin mediates activation of apoptosis signal-regulating kinase 1. Arch Biochem Biophys 415:133–136
43. Azad N, Vallyathan V, Wang L et al (2006) S-nitrosylation of Bcl-2 inhibits its ubiquitin-proteasomal degradation. A novel antiapoptotic mechanism that suppresses apoptosis. J Biol Chem 281:34124–34134

44. Qu J, Nakamura T, Cao G et al (2011) S-nitrosylation activates Cdk5 and contributes to synaptic spine loss induced by beta-amyloid peptide. Proc Natl Acad Sci USA 108:14330–14335
45. Tian J, Kim SF, Hester L et al (2008) S-nitrosylation/activation of COX-2 mediates NMDA neurotoxicity. Proc Natl Acad Sci USA 105:10537–10540
46. Westermann B (2009) Nitric oxide links mitochondrial fission to Alzheimer's disease. Sci Signal 2:pe29
47. Feng X, Sun T, Bei Y et al (2013) S-nitrosylation of ERK inhibits ERK phosphorylation and induces apoptosis. Sci Rep 3:1814
48. Yin XH, Yan JZ, Hou XY et al (2013) Neuroprotection of S-nitrosoglutathione against ischemic injury by down-regulating Fas S-nitrosylation and downstream signaling. Neuroscience 248C:290–298
49. Sen N, Hara MR, Ahmad AS et al (2009) GOSPEL: a neuroprotective protein that binds to GAPDH upon S-nitrosylation. Neuron 63:81–91
50. Koriyama Y, Chiba K, Yamazaki M et al (2010) Long-acting genipin derivative protects retinal ganglion cells from oxidative stress models in vitro and in vivo through the Nrf2/antioxidant response element signaling pathway. J Neurochem 115:79–91
51. Koriyama Y, Kamiya M, Takadera T et al (2012) Protective action of nipradilol mediated through S-nitrosylation of Keap1 and HO-1 induction in retinal ganglion cells. Neurochem Int 61:1242–1253
52. Gu Z, Kaul M, Yan B et al (2002) S-nitrosylation of matrix metalloproteinases: signaling pathway to neuronal cell death. Science 297:1186–1190
53. Fang J, Nakamura T, Cho DH et al (2007) S-nitrosylation of peroxiredoxin 2 promotes oxidative stress-induced neuronal cell death in Parkinson's disease. Proc Natl Acad Sci USA 104(47):18742–18747
54. Kim J, Won JS, Singh AK et al (2014) STAT3 regulation by S-nitrosylation: implication for inflammatory disease. Antioxid Redox Signal 20:2514–2527
55. Dawson TM, Chung KK (2009) S-nitrosylation of XIAP compromises neuronal survival in Parkinson's disease. Proc Natl Acad Sci USA 106:4900–4905
56. Koriyama Y (2013) Role of protein S-nitrosylation in central nervous system survival and regeneration. Yakugaku Zasshi 133:843–848
57. Koriyama Y, Takagi Y, Chiba K (2011) Neuritogenic activity of a genipin derivative in retinal ganglion cells is mediated by retinoic acid receptor β expression through nitric oxide/S-nitrosylation signaling. J Neurochem 119:1232–1242
58. Koriyama Y, Takagi Y, Chiba K (2013) Requirement of retinoic acid receptor β for genipin derivative-induced optic nerve regeneration in adult rat retina. PLoS One 8:e71252
59. Miller MR, Megson IL (2007) Recent developments in nitric oxide donor drugs. Br J Pharmacol 151:305–321
60. Adachi T, Hori S, Miyazaki K et al (1995) Rapid increase in plasma nitrite concentration following intravenous administration of nipradilol. Eur J Pharmacol 286:201–204
61. Taguchi R, Shirakawa H, Yamaguchi T et al (2006) Nitric oxide-mediated effect of nipradilol, an alpha- and beta-adrenergic blocker, on glutamate neurotoxicity in rat cortical cultures. Eur J Pharmacol 535:86–94
62. Nakazawa T, Tomita H, Yamaguchi K et al (2002) Neuroprotective effect of nipradilol on axotomized rat retinal ganglion cells. Curr Eye Res 24:114–122
63. Mizuno K, Koide T, Yoshimura M et al (2001) Neuroprotective effect and intraocular penetration of nipradilol, a beta-blocker with nitric oxide donative action. Invest Ophthalmol Vis Sci 42:688–694
64. Imai N, Tsuyama Y, Murayama K et al (1997) Protective effect of nitric oxide on ischemic retina. Nihon Ganka Gakkai Zasshi 101:639–643
65. Ando A, Yamazaki Y, Kaneko S et al (2005) Cytoprotection by nipradilol, an anti-glaucomatous agent, via down-regulation of apoptosis related gene expression and activation of NF-kappaB. Exp Eye Res 80:501–507

66. Tomita H, Nakazawa T, Sugano E et al (2002) Nipradilol inhibits apoptosis by preventing the activation of caspase-3 via S-nitrosylation and the cGMP-dependent pathway. Eur J Pharmacol 452:263–268
67. Naito A, Aniya Y, Sakanashi M (1994) Antioxidative action of the nitrovasodilator nicorandil: inhibition of oxidative activation of liver microsomal glutathione S-transferase and lipid peroxidation. Jpn J Pharmacol 65:209–213
68. Baird L, Dinkova-Kostova AT (2011) The cytoprotective role of the Keap1-Nrf2 pathway. Arch Toxicol 85:241–272
69. Himori N, Yamamoto K, Maruyama K et al (2013) Critical role of Nrf2 in oxidative stress-induced retinal ganglion cell death. J Neurochem 127:669–680
70. Eggler AL, Liu G, Pezzuto JM et al (2005) Modifying specific cysteines of the electrophile-sensing human Keap1 protein is insufficient to disrupt binding to the Nrf2 domain Neh2. Proc Natl Acad Sci USA 102:10070–10075
71. Kansanen E, Bonacci G, Schopfer FJ et al (2011) Electrophilic nitro-fatty acids activate NRF2 by a KEAP1 cysteine 151-independent mechanism. J Biol Chem 286:14019–14027
72. Li CQ, Kim MY, Godoy LC et al (2009) Nitric oxide activation of Keap1/Nrf2 signaling in human colon carcinoma cells. Proc Natl Acad Sci USA 106:14547–14551
73. Buckley BJ, Li S, Whorton AR (2008) Keap1 modification and nuclear accumulation in response to S-nitrosocysteine. Free Radic Biol Med 44:692–698
74. Charles I, Khalyfa A, Kumar DM et al (2005) Serum deprivation induces apoptotic cell death of transformed rat retinal ganglion cells via mitochondrial signaling pathways. Invest Ophthalmol Vis Sci 46:1330–1338
75. Nakajima Y, Shimazawa M, Otsubo K et al (2009) Zeaxanthin, a retinal carotenoid, protects retinal cells against oxidative stress. Curr Eye Res 34:311–318
76. Maines MD (1988) Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. FASEB J 2:2557–2568
77. Watanabe M, Tokita Y, Yata T (2006) Axonal regeneration of cat retinal ganglion cells is promoted by nipradilol, an anti-glaucoma drug. Neuroscience 140:517–528
78. Park KK, Liu K, Hu Y et al (2008) Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science 322:963–966
79. de Lima S, Koriyama Y, Kurimoto T et al (2012) Full-length axon regeneration in the adult mouse optic nerve and partial recovery of simple visual behaviors. Proc Natl Acad Sci USA 109:9149–9154
80. Koriyama Y, Kamiya M, Arai K et al (2014) Nipradilol promotes axon regeneration through S-nitrosylation of PTEN in retinal ganglion cells Adv Exp Med Biol 801:751–757
81. Liu K, Lu Y, Lee JK et al (2010) PTEN deletion enhances the regenerative ability of adult corticospinal neurons. Nat Neurosci 13:1075–1081