만성안압상승 쥐 모델에서 Sirtuin 1 기전을 통한 레스베라트롤의 신경보호 효과

Neuroprotective Effect of Resveratrol via Sirtuin 1 Pathway in a Rat Model of Chronic Ocular Hypertension

허 환, 성미선, 박상우

Hwan Heo, MD, Mi Sun Sung, MD, Sang Woo Park, MD, PhD

전남대학교 의과대학 안과학교실

Department of Ophthalmology, Chonnam National University Medical School, Gwangju, Korea

Purpose: To investigate the neuroprotective effect and to determine the neuroprotective mechanisms of resveratrol for retinal ganglion cells (RGC) in a rat model of chronic intraocular pressure (IOP) elevation.

Methods: Three-month-old Sprague-Dawley rats received saline or resveratrol (10 mg/kg) intraperitoneally once daily during the chronic IOP elevation period. Sirtuin 1 (SIRT1), peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1 α), and glial fibrillary acidic protein (GFAP) expression were assessed at 1, 2, 3, and 4 weeks following onset of chronic IOP elevation. The effects of resveratrol on SIRT1, PGC-1 α , and GFAP expression were assessed in retinas of rats treated with saline or resveratrol at 3 weeks after chronic IOP elevation. RGC survival was assessed by labeling flat-mounted retinas with Brn3a at 4 weeks after chronic IOP elevation.

Results: SIRT1 and PGC-1 α expression were downregulated in retinas from saline treated rats. This downregulation was reversed by resveratrol treatment, resulting in a significant difference between groups at 3 weeks following chronic IOP elevation. GFAP expression was upregulated in retinas of saline treated rats and suppressed by resveratrol treatment. Resveratrol treatment also protected against RGC loss at 4 weeks following chronic IOP elevation.

Conclusions: Our results highlight that resveratrol might be a potential neuroprotective agent against RGC loss via SIRT1 pathway under chronic IOP elevation conditions.

Key words: Neuroprotection, Sirtuin 1, Ocular hypertension

Several optic neuropathies, including glaucoma, lead to optic nerve axonal damage and retinal ganglion cell (RGC) loss via apoptosis.¹ Glaucomatous optic neuropathy is characterized by progressive axonal damage in the optic nerve head, which progresses to RGC death and visual field deficits.²

Since recognition of the promising role for silent information regulators in the longevity of *Caenorhabditis elegans*, many studies have focused on the cellular mechanisms underlying sirtuins.³ Sirtuin 1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD)⁺-dependent histone deacetylase, which is linked to cellular stress responses and survival functions including metabolic control, DNA repair, inflammation, apoptosis, and neuroprotection.^{4,5} Studies conducted to date indicate that SIRT1 contributes to neuroprotection during ischemia and in the neurodegenerative diseases.⁶⁻⁸ SIRT1 overexpression by resveratrol pretreatment mediates delayed neuronal death following cerebral ischemic damage in rats.⁹ In contrast, inhibition of SIRT1 increases cortical

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Corresponding Author: Sang Woo Park, MD, PhD
Department of Ophthalmology, Chonnam National University Medical School, #42 Jebong-ro, Dong-gu, Gwangju 61469, Korea
Tel: +82-62-220-6742, Fax: +82-62-227-1642
E-mail: exo70@naver.com

neuronal death after oxygen-glucose deprivation.¹⁰

Of several downstream targets of SIRT1 activation, regulation of peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1 α) activity plays a prominent role.¹¹⁻¹³ PGC-1 α is a transcriptional co-activator linked with mitochondrial biogenesis and function.¹⁴⁻¹⁶ Accumulating evidence indicates that abnormal regulation of PGC-1 α , which contributes to altered neuronal metabolism, occurs in neurodegenerative diseases.^{17,18}

Polyphenol resveratrol, a molecule containing two phenyl rings separated by a methylene bridge, was the first compound identified as a sirtuin stimulator.¹⁹ SIRT1 is present in the nucleus and cytoplasm of cells of all ocular structures, including the cornea, lens, iris, ciliary body, and retina.²⁰ Recently, Zuo et al.²¹ reported that SIRT1 overexpression or resveratrol treatment delayed RGC loss and loss of pupillary light responses by reducing superoxide production, following an optic nerve crush injury. Similarly, an anti-apoptotic effect of SIRT1 on cultured RGCs was reported in an in vitro model of hypoxia.²² In parallel, considering the wide spectrum of activities in which SIRT-1 participates, modulation of SIRT1 activity may have great importance in RGC fate in glaucomatous optic neuropathy. However, the neuroprotective effects of resveratrol on RGCs have not yet been reported in eyes with chronic intraocular pressure (IOP) elevation. Previously, our group reported that injection of 0.3% carbomer solution to the anterior chamber was an effective and reproducible method of producing chronic IOP elevation and progressive RGC death, including axonal loss, in rats.²³ The current study investigated the effects of resveratrol on RGC survival, including the mechanisms of action, in a rat model of chronic IOP elevation.

Materials and Methods

Animal Use

All experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The protocol was approved by the Institutional Animal Care and Use Committee of Chonnam National

University Hospital. Sprague-Dawley rats, each weighing 245-255 g, were individually housed under controlled lighting conditions (12 h light/12 h dark) and were given tap water and food ad libitum throughout the duration of the study.

Induction of IOP Elevation

IOP elevation was induced as described previously.²³ Briefly, anesthesia was induced in rats by intramuscular injection of a mixture of tiletamine/zolazepam (Zoletil 50, Virbac, Nice, France) 20 mg/kg combined with xylazine hydrochloride (Rompun, Bayer Korea, Seoul, Korea) 5 mg/kg. After tetracaine hydrochloride was topically applied, a sterile lid speculum was placed in the eye. The cornea was gently punctured near the limbus using a 31 gauge needle to decompensate the anterior chamber as much as possible prior to injection. After this entry wound, 20 μ L of 0.3% carbomer solution was injected into the anterior chamber using a 31-gauge needle. After each procedure, antibiotic ointment was applied. A topical antibiotic was applied twice each day, and eyes were observed for infection twice each day.

IOP Measurement

IOP was measured every day for 1 week and then weekly thereafter using a tonometer (TonoLab; Tiolat Oy, Helsinki, Finland) (Fig. 1). IOP measurements were conducted consistently at the same time in the afternoon. Rats were anaesthetized by intramuscular injection of tiletamine/zolazepam combined with xylazine hydrochloride. In order to minimize the effects of general anesthesia, IOP was measured as soon as the rat was lightly sedated and lid reflex was lost, which occurred within 1 minute. In total, 5 recordings were obtained from each eye, and the mean of these 5 recordings was calculated in order to determine IOP. Mean and peak IOP were measured at each time point. Integral IOP for each animal was defined as the area under the curve for each animal.²³

Pharmacological Treatment

Resveratrol (Sigma, St. Louis, MO, USA) was dissolved in 50% ethanol and diluted 10-fold in phosphate buffered

Figure 1. Schematic representation summarizing the study procedures. Arrows represent important procedural time points. IOP = intra-ocular pressure; SIRT1 = sirtuin 1; PGC-1 α = peroxisome proliferator-activated receptor gamma coactivator-1 α ; GFAP = glial fibrillary acidic protein; RGC = ganglion cells.

saline (PBS; pH 7.4). In order to evaluate the neuroprotective effects of resveratrol, rats were randomly divided into 2 experimental groups and injected intraperitoneally with saline (n = 65) or resveratrol (10 mg/kg/day; n = 25) daily until sacrifice.

Tissue Preparations

Rats were anesthetized as described above. Both eyes were enucleated, and rats were euthanized by CO₂ inhalation. The retinas were dissected from the choroid and fixed in 4% paraformaldehyde in PBS (pH 7.4) for 2 hours at 4°C for retinal flat mounting, or dehydrated in a graded series of ethanol solutions and then embedded in polyester wax. For western blot analyses, whole retinas were used immediately or frozen at -70°C until use.

Western Blot Analyses

Retinal tissues were homogenized in a glass-Teflon Potter homogenizer in lysis buffer (PRO-PREPTM, iNtRoN Biotechnology, Seoul, Korea). Each sample (10 μ g) was separated on a 10% polyacrylamide mini gel. After protein transfer, membranes were blocked for 1 hour at room tem-

perature in Tris-buffered saline Tween-20 solution (TBS-T; 10 mM Tris-HCl [pH 7.6], 150 mM NaCl, and 0.1% Tween-20) containing 5% non-fat dry milk. After blocking, membranes were incubated overnight at 4°C with a rabbit polyclonal anti-PGC-1 α antibody (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal anti-SIRT1 (1:1,000; Cell Signaling Technology, Danvers, MA, USA), mouse monoclonal anti-glial fibrillary acidic protein (anti-GFAP) (1:3,000; Cell Signaling Technology), or mouse monoclonal anti- β -actin antibody (1:4,000; Santa Cruz Biotechnology) in TBS-T solution containing 5% non-fat dry milk. After 3 washes with TBS-T, the membranes were incubated for 1 hour at room temperature with peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG) (1:3,000; Santa Cruz Biotechnology) or peroxidase-conjugated goat anti-rabbit IgG (1:3,000; Cell Signaling Technology) in TBS-T containing 5% nonfat dry milk. Signals were visualized by enhanced chemiluminescence and quantified using a LAS-3,000 image analyzer (Fujifilm, Tokyo, Japan).

Retinal Whole Mounts and Brn3a Staining

At 4 weeks after chronic IOP elevation, retinas were dis-

sected from enucleated eyes and flattened. Retinas were then immersed in PBS containing 30% sucrose for 24 hours at 4°C, then frozen for 15 minutes at -70°C, blocked in PBS containing 1% bovine serum albumin and 0.5% Triton X-100, and incubated with a polyclonal goat anti-brain-specific homeobox/POU domain protein 3a (Brn3a) antibody (1:100; Santa Cruz Biotechnology) for 72 hours at 4°C. After washing, the retinas were incubated with the secondary antibody, Alexa Fluor-568-conjugated donkey anti-goat IgG antibody (1:250; Invitrogen, Carlsbad, CA, USA) for 4 hours, and subsequently washed with PBS. To evaluate the loss of RGCs, each retinal quadrant was divided into 3 zones (center, middle, and peripheral retina) corresponding to 1/6, 3/6, and 5/6 of the retinal radius. RGCs were counted in 32 distinct areas of 0.09 mm² (2 areas in the center, 3 areas in the middle, and 3 areas at the periphery of each retinal quadrant) by 2 blinded investigators, and mean score was calculated. Images were analyzed using a fluorescence microscope (Nikon Eclipse E600; Nikon, Tokyo, Japan).

Immunohistochemical Analyses

Immunohistochemical staining of 7-μm wax sections of full-thickness retinas was performed by immunofluorescence with the following primary antibodies: mouse monoclonal anti-GFAP antibody (1:250; Cell Signaling Technology), mouse monoclonal anti-SIRT1 (1:100; Cell Signaling Technology), or goat polyclonal anti-Brn3a antibody (1:100;

Santa Cruz Biotechnology). Tissues were blocked with 1% bovine serum albumin in PBS for 1 hour at room temperature to prevent nonspecific background staining, and were then incubated with primary antibodies overnight at 4°C. The sections were washed several times, incubated with Alexa Fluor 488-conjugated chicken anti-mouse IgG (1:250; Invitrogen) or Alexa Fluor 546-conjugated rabbit anti-goat IgG (1:250; Invitrogen) for 4 hours at 4°C, and then washed again with PBS. The sections were counterstained with Hoechst 33342/PBS (0.1 μg/mL; Invitrogen). Images were analyzed using a Zeiss LSM 510 confocal microscope (Carl Zeiss, Jena, Germany).

Statistical Analyses

Experiments were repeated at least 3 times. The data presented represent means ± standard deviations. Groups were compared using the unpaired Student's *t*-test. Comparison between more than 2 conditions was performed using one-way analysis of variance and Bonferroni post-hoc multiple comparison tests. *p*-values < 0.05 were considered statistically significant.

Results

IOP Elevation

The mean IOP of control eyes and carbomer-injected eyes are described in Table 1. The mean IOP of the carbomer-in-

Table 1. The mean intraocular pressure at each follow-up period after injection of 0.3% carbomer solution

	Mean IOP (mmHg)			<i>p</i> -value*		
	Control	IOP + S	IOP + RV	P1	P2	P3
Baseline (n = 90)	9.60 ± 0.83	9.80 ± 1.21	9.93 ± 1.03	0.595	0.461	0.838
1 day (n = 90)	9.71 ± 1.27	18.86 ± 3.30	19.43 ± 3.52	< 0.001 [†]	< 0.001 [†]	0.701
3 days (n = 90)	10.46 ± 0.88	29.60 ± 5.70	29.40 ± 7.16	< 0.001 [†]	< 0.001 [†]	0.967
5 days (n = 90)	10.25 ± 1.60	32.13 ± 5.25	34.87 ± 4.52	< 0.001 [†]	< 0.001 [†]	0.255
7 days (n = 90)	9.80 ± 0.68	35.93 ± 6.94	36.71 ± 6.12	< 0.001 [†]	< 0.001 [†]	0.571
2 weeks (n = 80)	10.33 ± 1.18	40.08 ± 9.05	39.00 ± 8.89	< 0.001 [†]	< 0.001 [†]	0.478
3 weeks (n = 70)	10.73 ± 1.18	33.17 ± 9.24	33.50 ± 7.66	< 0.001 [†]	< 0.001 [†]	0.898
4 weeks (n = 30)	10.13 ± 0.83	24.42 ± 4.08	25.08 ± 6.43	< 0.001 [†]	< 0.001 [†]	0.977

Values are described as mean ± standard deviation. P1 Control vs IOP elevation + S, P2 Control vs IOP elevation + RV treatment, P3 IOP elevation + S vs IOP elevation + RV treatment.

IOP = intraocular pressure; S = saline; RV = resveratrol.

*Mann-Whitney *U* test for between-groups comparisons with Bonferroni correction for multiple comparisons; [†]*p* < 0.05.

Table 2. Integral intraocular pressure at each follow-up period after injection of 0.3% carbomer solution

	Integral IOP (mmHg)			<i>p</i> -value*		
	Control	IOP + S	IOP + RV	P1	P2	P3
1 week (n = 90)	80.38 ± 4.01	214.82 ± 29.78	223.29 ± 14.82	< 0.001 [†]	< 0.001 [†]	0.541
2 week (n = 80)	149.50 ± 5.58	471.88 ± 71.00	487.04 ± 42.60	< 0.001 [†]	< 0.001 [†]	0.410
3 week (n = 70)	221.83 ± 11.78	728.25 ± 110.46	740.79 ± 71.05	< 0.001 [†]	< 0.001 [†]	0.551
4 week (n = 30)	294.46 ± 13.80	929.79 ± 121.88	945.83 ± 92.94	< 0.001 [†]	< 0.001 [†]	0.590

Values are described as mean ± standard deviation. P1 Control vs IOP elevation + S, P2 Control vs IOP elevation + RV treatment, P3 IOP elevation + S vs IOP elevation + RV treatment. IOP = Intraocular pressure; S = Saline; RV = Resveratrol. *Mann-Whitney *U* test for between-groups comparisons with Bonferroni correction for multiple comparisons; [†]*p* < 0.05.

Figure 2. Expression of SIRT1, PGC-1α, and GFAP from rats experiencing chronic intraocular pressure (IOP) elevation. SIRT1 and PGC-1α expression was minimally downregulated at 3 weeks after chronic IOP elevation compared to control retinas (A, B, D, E) whereas GFAP expression was maximally upregulated at the same time point (C, F) (n = 10 retinas/group). The relative intensity of chemiluminescence for SIRT1, PGC-1α, and GFAP protein bands was normalized to β-actin. wk = week; SIRT1 = sirtuin 1; PGC-1α = peroxisome proliferator-activated receptor gamma coactivator-1α; IOP = intraocular pressure; GFAP = glial fibrillary acidic protein. *Significant at *p* < 0.05 compared to control. Error bars, standard deviation.

jected eyes remained significantly higher than control eyes until 4 weeks after carbomer injection, and reached a peak at 2 weeks after injection, with a mean IOP of 40.08 ± 9.05 mmHg in eyes from rats treated with saline and 39.00 ± 8.89 mmHg in eyes from rats treated with resveratrol (Table 1). The integral IOPs of the carbomer-injected eyes at 1, 2, 3, and 4 weeks after injection were significantly different from control eyes (Table 2). However, there was no significant difference in mean IOP and integral IOP in comparisons between carbomer-injected eyes from rats treated with saline and carbomer-injected eyes from the resveratrol

group at any experimental time point.

Expression of SIRT1, PGC-1α, and GFAP

The expressions of SIRT1, PGC-1α, and GFAP were evaluated at 1, 2, 3, and 4 weeks after chronic IOP elevation, using western blot analyses (Fig. 1). SIRT1 and PGC-1α expression were downregulated and reached a minimum at 3 weeks compared to control retinas without chronic IOP elevation (0.65 ± 0.13 -fold and 0.78 ± 0.14 -fold, respectively; *p* < 0.05) (Fig. 2A, 2B, 2D, 2E). In contrast, GFAP expression was upregulated and reached a maximum at 3 weeks

Figure 3. The effect of resveratrol on SIRT1 and PGC-1 α expression in retinas from rats experiencing chronic intraocular pressure (IOP) elevation. Resveratrol treatment significantly prevented the downregulation of SIRT1 and PGC-1 α expression compared with retinas from saline treated rats (A, B, C, D) ($n = 10$ retinas/group). Relative intensity of chemiluminescence for SIRT1 and PGC-1 α was normalized to β -actin. Counterstaining with Hoechst 33342 in a control retina (E, blue). Immunohistochemical staining with SIRT1 (F, green). Immunohistochemical staining with Brn3a, a marker for retinal ganglion cells (RGC; G, red). Merged image (H). These findings indicate that RGCs express SIRT1 protein (arrowheads). When the primary antibody was omitted (control for SIRT1 immunohistochemistry) there was no labeling by the secondary antibody in retinas from untreated rats (I). Compared to control retinas (J), SIRT1 immunoreactivity was decreased in retinas from rats with chronic IOP elevation (K). However, resveratrol treatment increased SIRT1 immunoreactivity in retinas from rats with chronic IOP elevation (L). SIRT1 = sirtuin 1; PGC-1 α = Peroxisome proliferator-activated receptor gamma coactivator-1 α ; IOP = intraocular pressure; S = saline; RV = resveratrol; GCL = ganglion cell layer; IPL = inner plexiform layer; RGC = ganglion cells. *Significant at $p < 0.05$ between control and chronic IOP elevation + saline. **Significant at $p < 0.05$ between chronic IOP elevation + saline and chronic IOP elevation + resveratrol. Error bars, standard deviation. Scale bar, 20 μ m.

compared to control retinas without chronic IOP elevation (1.48 ± 0.21 -fold; $p < 0.05$) (Fig. 2C, 2F).

Effect of Resveratrol on Expression of SIRT1, PGC-1 α , and GFAP

We evaluated whether resveratrol treatment affected the expression of SIRT1, PGC-1 α , and GFAP at 3 weeks after

chronic IOP elevation (Fig. 1). Resveratrol significantly prevented downregulation of SIRT1 expression compared to retinas from saline treated rats (0.66 ± 0.02 -fold vs. 0.95 ± 0.05 -fold; $p < 0.05$, $n = 10$ retinas/group) (Fig. 3A, 3C). A similar effect of resveratrol on PGC-1 α expression was evident when compared to retinas from saline treated rats (0.87 ± 0.02 -fold vs. 1.08 ± 0.02 -fold; $p < 0.05$) (Fig. 3B,

Figure 4. The effect of resveratrol on GFAP expression in retinas from rats experiencing chronic intraocular pressure (IOP) elevation. Resveratrol treatment significantly suppressed the upregulation of GFAP expression compared retinas from saline treated rats (A, B; $n = 10$ retinas/group). Relative intensity of chemiluminescence of GFAP protein bands was normalized to β -actin. When the primary antibodies were omitted (control for GFAP immunohistochemistry), there was no secondary antibody binding (C). Compared with control retinas (D), GFAP immunoreactivity was increased in retinas from saline treated rats with chronic IOP elevation (E). However, resveratrol treatment decreased GFAP immunoreactivity in retinas from rats with chronic IOP elevation (F; $n = 5$ retinas/group). GFAP = glial fibrillary acidic protein; IOP = intraocular pressure; S = saline; RV = resveratrol; GCL = ganglion cell layer; IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer; ONL = outer nuclear layer. *Significant at $p < 0.05$ between control and chronic IOP elevation + saline. **Significant at $p < 0.05$ between chronic IOP elevation + saline and chronic IOP elevation + resveratrol. Error bars, standard deviation. Scale bars, 20 μ m.

3D). In contrast, resveratrol significantly suppressed GFAP upregulation in comparison with retinas from saline treated rats (1.31 ± 0.08 -fold vs. 1.00 ± 0.04 -fold; $p < 0.05$) (Fig. 4A, 4B).

The ganglion cell layer (GCL) was visualized using Hoechst 33342 (Fig. 3E), SIRT1 (Fig. 3F), and Brn3a (Fig. 3G) staining. Upon double SIRT1 and Brn3a immunohistochemical staining (Fig. 3H), GCL neurons positive for SIRT1 were co-immunostained for Brn3a, an RGC marker. Indicating that RGCs express SIRT1. In the absence of the primary SIRT1 antibody, there was no secondary antibody labeling in untreated rat retinas (negative control) (Fig. 3I). SIRT1 immunoreactivity was reduced in the GCL of saline treated retinas compared to retinas from untreated

rats without chronic IOP elevation (Fig. 3J, 3K). Resveratrol treatment increased SIRT1 immunoreactivity in retinas with chronic IOP elevation (Fig. 3L). Without primary GFAP antibody, there was no labeling by the secondary antibody in retinas from untreated rat (negative control) (Fig. 4C). GFAP immunoreactivity was increased in the nerve fiber layer and GCL of retinas from saline treated rats compared to control retinas ($n = 5$ retinas/group) (Fig. 4D, 4E). Moreover, resveratrol treatment decreased GFAP immunoreactivity in retinas (Fig. 4F).

Effect of Resveratrol on RGC Loss after Chronic IOP Elevation

RGC survival was evaluated by immunohistochemistry of retinal whole mounts using an anti-Brn3a antibody. Signif-

Figure 5. The effect of resveratrol on retinal ganglion cell (RGC) survival in retinas from rats experiencing chronic intraocular pressure (IOP) elevation. Retinal flat mounts are shown for control (A), control-resveratrol (B), chronic IOP elevation+saline (C), and chronic IOP elevation+resveratrol groups (D) ($n = 10$ retinas/group). Quantitative analysis of RGC survival (E). RGC = retinal ganglion cell; IOP = intraocular pressure; S = saline; RV = resveratrol. *Significant at $p < 0.05$ between control and chronic IOP elevation + saline. **Significant at $p < 0.05$ between chronic IOP elevation + saline and chronic IOP elevation + resveratrol. Error bars, standard deviation. Scale bars, 20 μm .

Table 3. The effect of resveratrol on retinal ganglion cell survival in retina with chronic IOP elevation

	Cell density (cell/mm ² $\pm$ SD)			
	Control	Control + RV	IOP + RV	IOP + RV
Center	2,128 $\pm$ 556	2,191 $\pm$ 556	1,373 $\pm$ 394*	2,033 $\pm$ 398 [†]
Middle	1,903 $\pm$ 534	1,995 $\pm$ 552	1,174 $\pm$ 418*	1,851 $\pm$ 565 [†]
Periphery	1,562 $\pm$ 609	1,542 $\pm$ 573	897 $\pm$ 370*	1,378 $\pm$ 518 [†]

Values are described as mean $\pm$ standard deviation.

IOP = intraocular pressure; S = saline; RV = resveratrol.

* $p < 0.05$, comparison between control and IOP elevation + S; [†] $p < 0.05$, comparison between IOP elevation + S and IOP elevation + RV.

Significant differences in RGC density occurred between retinas from resveratrol treated rats and those from saline treated rats at 4 weeks after chronic IOP elevation. The saline group experienced significant RGC losses of approximately 36% in the center, 39% in the middle, and 43% in the periphery, compared to the control group without chronic IOP elevation. Resveratrol treatment prevented RGC loss by ap-

proximately 48% in the center, 57% in the middle, and 54% in the periphery compared to retinas from saline treated rats ($p < 0.05$, $n = 10$ retinas/group) (Fig. 5, Table 3). These results suggest that resveratrol has a neuroprotective effect on RGCs in conditions of chronic IOP elevation.

Discussion

RGC death in optic neuropathies, including glaucoma, are thought to occur via several mechanisms, such as interruption of retrograde neurotrophin transport, mitochondrial dysfunction, oxidative stress, and excitotoxicity.^{2,24-26} Under these harmful conditions, RGCs may undergo apoptosis, but they can potentially be rescued by neuroprotective agents. To date, however, there is no effective treatment to prevent RGC death in diverse optic neuropathies. Resveratrol is a natural polyphenolic compound mainly found in grape skins.²⁷ Resveratrol significantly increases SIRT1 activity through an allosteric interaction, resulting in an increase in SIRT1 affinity for both NAD⁺ and the acetylated substrate.¹⁹ Moreover, SIRT1 physically interacts with and deacetylates PGC-1 α at multiple lysine sites, consequently increasing PGC-1 α activity.²⁸ PGC-1 α is a transcriptional co-activator implicated in mitochondrial biogenesis and respiration through the regulating several genes, such as nuclear respiratory factors and mitochondrial transcription factor A.^{15,29,30} Emerging evidence has indicates that SIRT1 is an important anti-aging molecules and may participate in preventing several age-related ocular diseases.³¹⁻³³ In addition, several neurodegenerative diseases including Huntington's disease and Alzheimer's disease are associated with reduced SIRT1 expression.^{34,35} In the retina, the neuroprotective effects of SIRT1 may be regulated by inhibiting oxidative stress-related retinal damage, apoptosis-related retinal death, and inflammation.^{32,36,37}

Increased PGC1 α expression is implicated in elevated mitochondrial biogenesis and efficient respiration, in parallel with elevated expression of mitochondrial reactive oxygen species (ROS) detoxifying enzymes.^{38,39} In neurons, mitochondria play important roles in the maintenance of cellular homeostasis, including adenosine triphosphate (ATP) production by oxidative phosphorylation, maintaining intracellular calcium for controlling neuronal excitability, ROS production, and regulation of death signaling pathways.^{40,41} Previous studies have suggested that mitochondrial dysfunction contributes to RGC death in cultured RGC-5 cells, as well as in ischemic and glaucomatous animal models.⁴²⁻⁴⁶

Similarly, our group previously demonstrated that ischemia reperfusion injury by acute IOP elevation increases expression of a dynamin related guanosine triphosphate (GTP)ase (dynamin-related protein 1 [Drp-1]), which plays a key role in mitochondrial fragmentation at the onset of apoptosis in ischemic mouse retinas. Furthermore, the neuroprotective dipeptide carnosine significantly decreases RGC loss by lowering Drp-1 expression in ischemic mouse retinas.⁴⁷ Increased GFAP expression is commonly interpreted as a cellular marker of retinal stress and astrogliosis.⁴⁸ This augmented expression suggests increased stress in retinas with chronic IOP elevation, and thus these findings can be interpreted as evidence that resveratrol treatment exerts a protective effect during harmful stress, which results in RGC survival. Moreover, resveratrol ameliorates mitochondrial dysfunction in retinas with chronic IOP elevation.

In conclusion, resveratrol treatment decreases RGC death by upregulating SIRT1 and PGC-1 α expression and by downregulating GFAP expression in rat retinas with chronic IOP elevation. Resveratrol has multiple avenues by which it maintains cellular homeostasis. Therefore, we believe that the neuroprotective effect of resveratrol on RGCs is not limited to one factor, rather involves multiple mechanisms, most of which may be interrelated. The neuroprotective effects of resveratrol combined with its minimal side effects make it a promising novel neuroprotective treatment for optic neuropathies, including glaucoma. Precise delineation of resveratrol's pharmacologic mechanisms and dosing regimens are required in order for preclinical results to develop into future treatments in clinical practice.

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