Effect of Resveratrol on Sirtuins, OPA1, and Fis1 Expression in Adult Zebrafish Retina

Weiwei Sheng,[1,2] Ye Lu,[1] Feng Mei,[1] Ning Wang,[1] Zhi-Zhi Liu,[3–5] Ying-Ying Han,[3–5] Han-Tsing Wang,[3–5] Suqi Zou,[3,4] Hong Xu,[3–5] and Xu Zhang[1,5]

[1]Affiliated Eye Hospital of Nanchang University, Jiangxi Research Institute of Ophthalmology and Visual Science, Nanchang, China
[2]Queen Mary School of Nanchang University, Nanchang, China
[3]Institute of Life Science of Nanchang University, Nanchang, China
[4]School of Life Sciences of Nanchang University, Nanchang, China
[5]Jiangxi Provincial Collaborative Innovation Center for Cardiovascular, Digestive and Neuropsychiatric Diseases, Nanchang, China

Correspondence: Xu Zhang, Affiliated Eye Hospital of Nanchang University, 463 Bayi Road, Nanchang, Jiangxi 330006, China; xuzhang19@163.com.
Hong Xu, Institute of Life Science, Nanchang University, Nanchang 330031, China; xuhong@ncu.edu.cn.

WS, YL, and FM contributed equally to the work presented here and should therefore be regarded as equivalent authors.

Submitted: April 10, 2018
Accepted: August 7, 2018

Citation: Sheng W, Lu Y, Mei F, et al. Effect of resveratrol on sirtuins, OPA1, and Fis1 expression in adult zebrafish retina. *Invest Ophthalmol Vis Sci*. 2018;59:4542–4551. https://doi.org/10.1167/iovs.18-24539

Purpose. We determined whether sirtuins (SIRT1-SIRT7) are expressed in the zebrafish retina, evaluated the modulatory effect of resveratrol in the normal retina, and examined N-Methyl-D-aspartic acid (NMDA)-induced zebrafish retinal damage associated with mitochondrial sirtuins and mitochondrial fusion and fission mediators, OPA1 and Fis1.

Methods. Sirtuins, OPA1, and Fis1 mRNA expression was analyzed by RT-PCR and quantitative real time PCR (qPCR) in adult zebrafish (AB type) retina and liver. qPCR showed an effect of resveratrol on SIRTs (SIRT1, 3, 4, 5) and OPA1 and Fis1 in low and high concentrations (5 and 50 mg/L) at different time points (0, 1, 24, and 48 hours) in the retina. Western blots were performed to examine the expression of SIRTs and OPA1 proteins under high concentrations of resveratrol for 24 hours. Hematoxylin and eosin staining, qPCR and mitochondrial copy number, and DNA damage assays then were used to confirm the protective effects of resveratrol on NMDA-induced retinal damage.

Results. The seven sirtuins and OPA1 were highly expressed in zebrafish retina compared to the liver. Treatment with resveratrol promoted SIRT1, mitochondrial sirtuins, and *OPA1* gene and protein expression, and improved mitochondrial DNA repair in adult zebrafish retina. Interestingly, the effect of resveratrol on *SIRT4* gene and protein expression was significantly higher in the zebrafish retina. Importantly, resveratrol offered protection against NMDA-induced retinal damage by activating the *SIRT1* gene and subsequent protein expression. Mitochondrial sirtuins and *OPA1* genes likely had a role in regulating mitochondrial dynamics.

Conclusions. To our knowledge, our study is the first composite analysis of sirtuins in adult zebrafish retina and provides sufficient evidence that resveratrol, as an activator of SIRT1, protects NMDA-induced zebrafish retinal damage by potentially mediating mitochondrial sirtuins and *OPA1* genes.

Keywords: zebrafish, retina, resveratrol, sirtuins, mitochondria, OPA1, NMDA

Glaucoma, a progressive optic neuropathy, is due to increased IOP, which causes oxidative stress in the retina and optic nerve head and also induces apoptosis.[1] Mitochondrial dysfunction can arise from a variety of potential injuries, such as oxidative injury, mechanical stress, aging, and others in glaucoma.[2] However, research on the underlying mechanisms of glaucoma and potential drug targets is poorly understood. At present, treatments for glaucoma still aim to reduce IOP, while enhancing neuroprotective effects receive less attention.

Resveratrol is a natural polyphenol derived from edible plants and herbs, such as *Polygounm cuspidatum*, mulberry, peanut, and grape skins and seeds, and is found mainly in red wine.[3,4] Many studies have found significant effects of resveratrol in the treatment of various diseases, such as cardiopathy,[4] autoimmune diseases,[5] neurodegeneration,[6] obesity, diabetes,[7] and some cancers.[8,9] Growing evidence has indicated that resveratrol leads to potent activation of SIRT1, which can inhibit cellular oxidative stress in the diabetic milieu, prevent retinal detachment, and mitigate rat retinal ischemic injury.[10–12] Nevertheless, the relationship between these effects and other SIRTs remains unknown. SIRT3, SIRT4, and SIRT5 all are localized in mitochondria.[13] Neuronal SIRT3 protects against excitotoxic injury in mouse cortical neuron.[14] Additionally, SIRT3 also protects mitochondria from oxidative damage, by deacetylating FOXO3, and against damage from mitochondria-derived reactive oxygen species (ROS) that are produced from complex I and complex III.[15,16] Sirt4 interacts with optic atrophy 1 (OPA1) and regulates mitochondrial quality control and mitophagy.[17] SIRT5 has demonstrated important neuroprotective effects.[18] Fission protein 1 (Fis1) and OPA1 proteins are important in mediating mitochondrial membrane fission and fusion.[19,20] OPA1 mutations reportedly can cause optic atrophy in retinal ganglion cell (RGC) pathophysiology.[21] Therefore, it is of great interest to understand how resveratrol modulates SIRTs and mitochondrial proteins (OPA1 and Fis1) in the retina.

The zebrafish (*Danio rerio*) has become known as a powerful model organism in biomedical research.[22] The small

Copyright 2018 The Authors

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

Downloaded from iovs.arvojournals.org on 04/24/2024

animals are easy to maintain and manipulate, low cost, and can lay hundreds of eggs at weekly intervals, and drug administration is quick and simple.[23,24] Additionally, approximately 70% to 80% of zebrafish genes share homology with the human genome.[25] Zebrafish bioassays also are cheaper and faster than mouse assays, and can be used to assess toxicity responses, teratogenic effects, and LC(50).[26] Over the past decade, researchers have started using zebrafish models to study different ophthalmologic disorders, such as cataracts, glaucoma, diabetic retinopathy, and age-related macular degeneration (AMD).[24] For example, the zebrafish mutants (lamb and pax2) reveal the potential of aminoglycosides in the treatment of human coloboma.[27] Furthermore, hypoxic zebrafish models have been used to test oral antiangiogenesis inhibitors to rescue neovascularization of the retina.[28] Therefore, zebrafish is a promising model that can be used to investigate the action mechanisms of antiglaucoma drugs and search for new drug targets.

We investigated three questions to evaluate the modulatory effect of resveratrol in the zebrafish retina. First, are SIRTs and mitochondrial (*OPA1*, *Fis1*) genes expressed in wild-type adult zebrafish retina? Second, what is the effect of resveratrol on SIRTs (SIRT1, 3, 4, 5) and mitochondrial gene (*OPA1* and *Fis1*) expression in the adult zebrafish retina? Finally, does resveratrol have a neuroprotective effect in NMDA-induced retinal damage in adult zebrafish?

Materials and Methods

Animals

Both sexes of 3- to 6-month-old wild-type zebrafish (*Danio rerio*) of AB strain were obtained from the China Zebrafish Resource Center, CZRC (Wuhan, China). All animals were fed brine shrimp twice a day in a 25 L aquarium, and the temperature was maintained at 28.5°C under a 14-hour/10-hour light/dark cycles. The use and manipulations of zebrafish were approved by the ethical review committee of Nanchang University (Nanchang, China) and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Primer Design

The sequences of SIRTs, OPA1, and Fis1 were identified in zebrafish using the NCBI-BLAST search of GeneBank, with a subsequent search for cDNAs. Primers for the zebrafish genes were designed by primer 3 software (Table 1).

Resveratrol Exposure

Untreated wild-type adult zebrafish were used as the control group. For the resveratrol-treated group, experiments were performed as described previously using zebrafish liver.[23,29] Resveratrol (R5010; Sigma-Aldrich Corp., St. Louis, MO, USA) was dissolved in ethanol and had a final storage solubility of 50 mg/L. A preliminary study was performed to verify whether zebrafish retina showed certain gene expression profiles. For resveratrol exposure, the animals were transferred to water containing 50 mg/L resveratrol for 1, 24, or 48 hours for complete solubilization. The control groups, which were exposed only to ethanol, also were treated for 1, 24, and 48 hours. To test the effects of low resveratrol concentrations (5 mg/L), control and treatment groups were kept for 1 and 24 hours. Resveratrol is degraded when exposed to ultraviolet light, so all fish were treated in a dark environment at 28.5°C (Fig. 1).

Table 1. PCR Primers Used in the Study

Genome	Primers (5′ to 3′)
SIRT1	F: CAAGGAAATCTACCCCGGACAGT
	R: CAGTGTGTCGATATTCTGCGTGT
SIRT2	F: ATCCCAGATTTCCGCTCTCC
	R: AGCGCCTCAGTAAGCCTTTG
SIRT3	F: CCTTTGTAATCCCTCCGACAAAC
	R: CATGCTTCACGATAAGGAACAGC
SIRT4	F: GATGGTCAGCTCGTGTAGCTCC
	R: GTGGTCAAGCGGAATACAGTTCA
SIRT5	F: CAGCTCATACAGCGGGTTTTAAAC
	R: CCTGAAGCTTTTTCTCGAGATC
SIRT6	F: ACACGGTGGTCGGAGTGATG
	R: TCCTCCTGTTCGTTTGGTCA
SIRT7	F: CAACGGCAGACTATGAAGAC
	R: CTGATTCCTGCTCCGGTGTA
OPA1	F: GCTTGAGCGCTTGGAAAAGGAA
	R: TGGCAGGTGATCTTGAGTGTTGT
Fis1	F: CTAGCTCCAGGGCCTGTTTGT
	R: GGTGAAAGGACCCGTTTCCAG
β-actin	F: CCCAAGGCCAACAGGGAAAA
	R: GGTACGACCGGAGGCATACA
Nuclear target (233 bp)	F: ATGGGCTGGGCGATAAAATTGG
	R: ACATGTGCATGTCGCTCCCAAA
Mitochondrial target (10.3 kb)	F: TTAAAGCCCCGAATCCAGGTGAGC
	R: GAGATGTTCTCGGGTGTGGGATGG
Mitochondrial target (198 bp)	F: CAAACACAAGCCTCGCCTGTTTAC
	R: CACTGACTTGATGGGGGAGACAGT

Drug Treatment

All chemical treatments were used in a 0.1% ethanol solution to improve drug uptake in 250 mL distilled water to ensure complete solubilization. A preliminary study was performed to verify what concentration of N-methyl-D-aspartate (NMDA; M3262; Sigma-Aldrich Corp.) could induce neurologic insult. A significant effect was noted with a 100 μM concentration of NMDA compared to the effect observed in controls (data not shown). The fish were classified into four treatment groups (at least four fish in each group): (1) Control group, (2) 50 mg/L resveratrol, (3) 100 μM NMDA, and (4) 100 μM NMDA+ 50 mg/L resveratrol. All groups were incubated at 28.5°C for 24 hours in a dark environment. At the end of the treatment, zebrafish retinas and livers were dissected and immediately frozen in liquid nitrogen for later analysis.

Reverse Transcription PCR

Retinas ($n = 8$ per group) were harvested, total RNA was extracted with TRIzol Reagent (Cat. 92008; Ambion, Austin, TX, USA) in accordance with the manufacturer's instructions. RNA was quantified by spectrophotometry, the ratio of A260/A280 is approximately 1.9 (>1.8), and all samples were adjusted to 2 μg/mL. cDNA species were synthesized with TIANscript RT kit (Tiangen, Beijing, China), which is a synthesis system for RT-PCR, according to the manufacturer'-seconds instructions. PCR cycles was performed with the following conditions: denatured, 95°C, 30 seconds; annealing, 58°C, 30 seconds; and extension, 72°C, 15 seconds, total 37 cycles. The reactions were performed to amplify each primer pair (Table 1). Low DNA mass ladder (Takara, Beijing, China) was used as a molecular maker. The amplified products were visualized on a 1.0% agarose gel with ethidium

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 04/24/2024

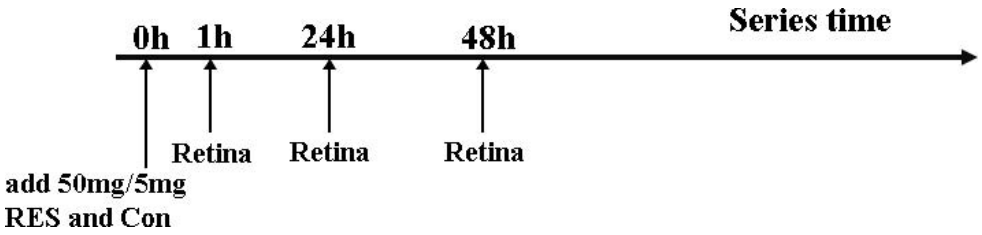

FIGURE 1. Methodological timeline. All treatments began at 0 hour and the time points are postresveratrol administration. Con, 0.1% ethanol; 5 mg RES, 5 mg/L resveratrol+0.1% ethanol; 50 mg/L RES, 50 mg/L resveratrol+0.1% ethanol.

bromide under ultraviolet light by Image Lab 5.2.1 for windows.

Real Time Quantitative PCR (qPCR)

Retinas ($n = 8$ per group) and livers ($n = 4$ per group) were harvested, total RNA was extracted. The cDNA products were used as a template for qPCR. qPCR was performed using the Quant One Step qRT-PCR Kit (SYBR Green; Takara), according to the manufacturer's seconds instructions. Three-step PCR with a 60°C annealing temperature was used for all primers (Table 1). Relative gene expression was quantified using the StepOne Plus TM Real-time PCR System (Life Technologies, Carlsbad, CA, USA).

Western Blot Analysis

Retinal tissues ($n = 8$ retinas/group) were lysed by radioimmunoprecipitation assay (RIPA) buffer (R0010; Solarbio, Beijing, China). The concentration of protein was determined using an enhanced BCA Protein Assay Kit (P0010S, Beyotime Biotechnology, Shanghai, China). We used 30 μg total protein lysate for the Western blot analysis and each sample (10 μg) was separated by PAGE and electrotransferred to polyvinylidene difluoride membranes. We then used appropriate primary antibodies (Table 2) and incubated the membranes. The secondary antibodies included goat anti-mouse, rabbit anti-goat or goat anti-rabbit antibodies (1:2500, ZSGB-BIO, Beijing, China). Bands were exposed with a SYNGENE image system and quantified using ImageJ (National Institutes of Health, Bethesda, MD, USA).

HE Staining and Histologic Evaluation

Fish were anesthetized in 0.03% tricaine methane-sulfonate (MS-222, Sigma-Aldrich Corp.). Eyeballs from adult zebrafish were fixed in 4% paraformaldehyde in PBS at 4°C for 5 hours. Following washing in 1× PBS three times at 4°C for 30 minutes, the eye tissue was dehydrated in 30%, 50%, and 70% ethanol sequentially and at 4°C for 30 minutes. All dehydrated eyeballs were embedded in paraffin blocks. All sections were cut to 4 μm and obtained using a Manual Rotary Microtome (RM2235; Leica, Wetzlar, Germany). Retinal sections were stained with hematoxylin and eosin (HE).

For histology analysis, 10 eyeballs of each group were used for item measurement. Ten retinal sections of each eyeball were used to measure average values for each eyeball. All slides were selected from the central area of the eyeballs through the optic nerve. The thickness of nerve fibers and ganglion cell layers was measured using Image-pro Plus 6.0 in a region beginning 45 μm from the center of the optic nerve head and ending 80 μm from the center of the optic nerve head (retinal length, 125 μm).

Mitochondrial Copy Number and DNA Damage Assays

Retinal DNA was extracted using the Ezup column animal genomic DNA extraction kit (Sangon Biotech, Shanghai, China). Long and short mitochondrial segments were amplified from 15 ng total DNA using the Long Amplification Taq polymerase kit (Takara). Long-mitochondrial-segment primers (10.3 kb), short-mitochondrial-segment primers (198 bp) (Table 1) and all reaction conditions were used as previously described using zebrafish cells.[30] Long-segment conditions were as follows: 19 cycles (94°C for 15 seconds; 68°C for 12 minutes) with a final extension step of 72°C for 10 minutes. Short-segment conditions were as follows: 25 cycles (94°C for 30 seconds; 62°C for 45 seconds; 72°C for 30 seconds) with a final extension step of 72°C for 10 minutes. All long- and short-segment reactions were stopped in the linear phase. Low DNA mass ladder (Takara) and DNA Ladder P (250∼10000 base pairs [bp]; Takara) were used as a molecular maker. The amplified products were visualized on a 0.8% agarose gel with ethidium bromide under ultraviolet light by Image Lab 5.2.1 for windows. Bands were then quantified by ImageJ.

Retinal mitochondrial copy number (mitCN) was estimated by qPCR for total DNA extracted using Ezup column animal genomic DNA extraction kit (Sangon Biotech). Primer sequences for the mitochondrial segment (198 bp) and nuclear segment (233 bp) were as previously described[30] (Table 1).

TABLE 2. Primary Antibodies Used in the Study

Antibody	Source	Catalog No.	Type of Ab	Dilution	MW, kD
SIRT1	Sigma	s5447	Rabbit mAb	1:1000	120
SIRT3	Abcam	86671	Rabbit mAb	1:1000	36.6
SIRT4	Abcam	10140	Goat polyclonal	1:1000	36
SIRT5	Abcam	78982	Rabbit polyclonal	1:1000	34
OPA1	BD	612606	Mouse mAb	1:1000	80–100
β-Actin	TRANS	HC201	Mouse mAb	1:1000	42

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 04/24/2024

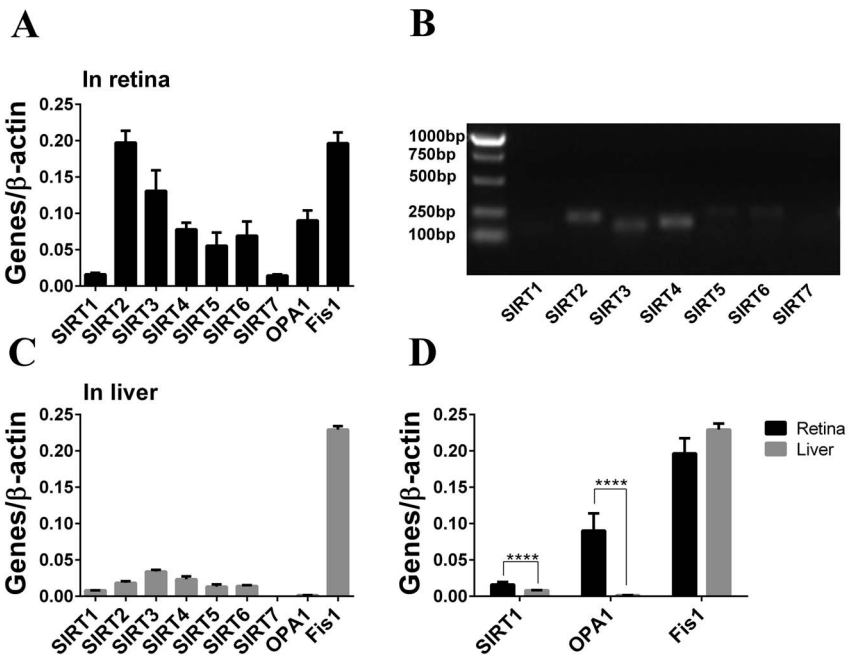

Figure 2. SIRTs and mitochondrial gene (*OPA1* and *Fis1*) expression in normal wild-type zebrafish retina and liver. (**A**) Graphic representation of changes in *SIRTs*, *OPA1*, and *Fis1* gene expression by real-time PCR in zebrafish retina. *Error bars*: SEM; $n = 6$ for qPCR. (**B**) The *leftmost band* is the DNA marker. The representative gel of semi-quantitative RT-PCR experiments show that *sirtuins* genes have different band densities in the adult zebrafish retina. (**C**) Graphic representation of changes in *SIRTs*, *OPA1*, and *Fis1* gene expression by real-time PCR in zebrafish liver. *Error bars*: SEM; $n = 6$ for qPCR. (**D**) SIRT1, OPA1, and Fis1 mRNA levels in the retina compared to the liver were measured by real-time PCR. Data represent the ratio of the mRNA level in liver tissue to that in the retina. Each value represents the mean ± SEM ($n = 6$). Unpaired *t*-test, $^{****}P < 0.0001$.

MitCN was calculated relative to nuclear DNA using the following equations:[31]

$$\Delta C_T = \text{mitochondrial } C_T - \text{nuclear } C_T \quad (1)$$

$$\text{Relative mitochondrial DNA content} = 2 \times 2^{-\Delta CT} \quad (2)$$

Statistical Analysis

Data processing and statistical analysis were performed using Microsoft Excel and Prism 6. The results were expressed as mean ± SEM and used nonparametric *t*-tests, considering $P \leq 0.05$ as statistically significant. The variance also was analyzed.

Results

SIRTs and Mitochondrial Gene (*OPA1* and *Fis1*) Expression in Normal Wild-Type Zebrafish Retina

We first analyzed the mRNA levels of SIRTs in normal wild-type zebrafish retina (Figs. 2A, 2B). The SIRTs mRNA levels varied depending on tissue type. mRNA level of SIRT2 was highest in the retina, with lower levels in the liver. SIRT1 and SIRT7 were lower than the other SIRTs in the retina and liver (Figs. 2A–C). mRNA levels of SIRT3, SIRT4, and SIRT5 were found in descending order, respectively, in the retina and liver. These mitochondria-related genes made up a large part of the SIRT family. The retina and liver are high energy-consuming tissues. Fis1 mRNA was highly expressed in the retina and liver, while OPA1 was expressed at higher levels in the retina than in the liver (Fig. 2D). Importantly, we found that all seven SIRTs were highly expressed in the retina when compared to the liver.[32]

Effect of Resveratrol on SIRTs (SIRT1, 3, 4, 5) and Mitochondrial Gene (*OPA1* and *Fis1*) Expression in Adult Zebrafish Retina

Prior studies have suggested that resveratrol does not alter mRNA level of the *SIRT1* gene, but negatively regulates *SIRT3* and *SIRT4* gene expression in zebrafish liver following exposure to resveratrol (5 and 50 mg/L) for 30 and 60 minutes.[23] We also found *SIRT1* gene expression does not change after exposure to 50 mg/L resveratrol for 24 hours, while *SIRT3* gene expression still decreases in the zebrafish liver (Supplementary Figs. S1A–B). However, mRNA level of SIRT4 increases after treatment with resveratrol for 24 hours (Supplementary Fig. S1C). We observed that resveratrol highly regulates *SIRTs* gene expression in the retina when compared to the liver (Fig. 4; Supplementary Fig. S1). Additionally, we found the resveratrol increased *SIRT1* gene and protein expression in the zebrafish retina (Figs. 3A, 4A, 4G, 4H). Low and high concentrations of resveratrol (5 and 50 mg/L) could increase SIRTs (SIRT1, 3, 4, and 5) and mitochondrial gene (*OPA1* and *Fis1*) expression. As time goes on, the effect of the resveratrol on SIRTs and mitochondrial genes expression increased initially, but was then followed by a decrease (Figs. 3A–F, 4A–F).

After exposure to low concentrations of resveratrol, SIRTs (SIRT1, 3, 4, 5) and mitochondrial gene expression peaked by the 1-hour time point (Figs. 3A–F). However, exposure to high concentrations of resveratrol resulted in SIRTs and mitochondrial gene expression increasing at the 1-hour mark, peaking by 24 hours, and decreasing at 48 hours (Figs. 4A–F). In addition, Western blot analysis showed that SIRT3, 4, 5 and OPA1 protein expression significantly increased at 24 hours (Figs. 4H, 4J). High concentration of resveratrol significantly altered SIRTs and mitochondrial gene expression when compared to the low concentration. The effect of resveratrol on *SIRT4* gene

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 04/24/2024

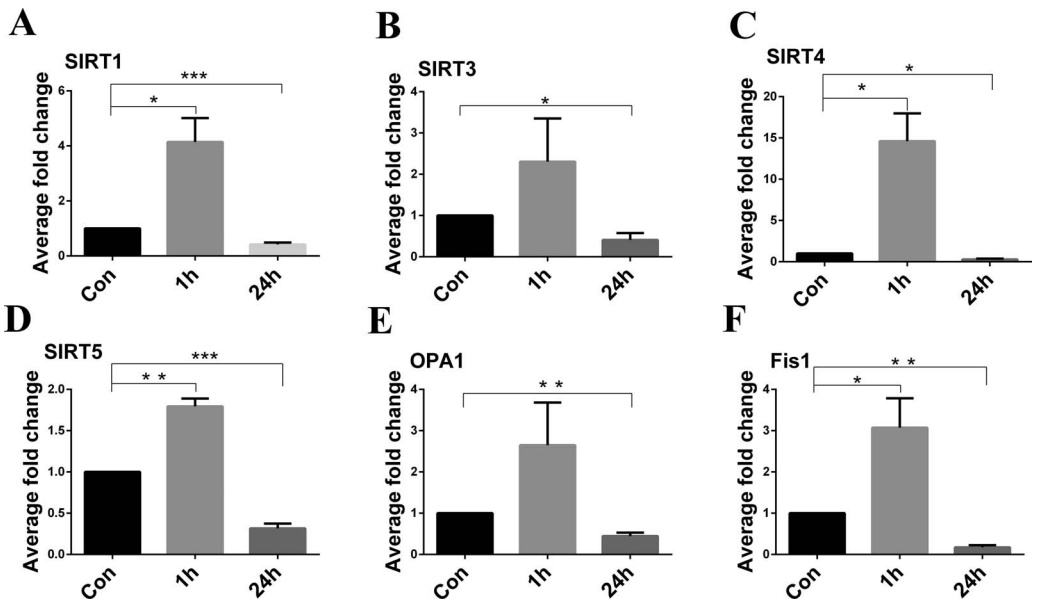

FIGURE 3. Effect of low concentration of resveratrol on SIRT and mitochondrial gene (*OPA1* and *Fis1*) expressions in adult zebrafish retina. (**A–F**) Gene expression in zebrafish retina after exposure to 5 mg/L resveratrol for 1 and 24 hours. Graphic representation of average fold changes in (**A**) *SIRT1*, (**B**) *SIRT3*, (**C**) *SIRT4*, (**D**) *SIRT5*, (**E**) *OPA1*, and (**F**) *Fis1* gene expression by qRT-PCR. *Error bars*: SEM; $n = 4$ for qPCR. (unpaired *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared to control (Con).

and protein expression was significantly higher in the zebrafish retina (approximately 15-fold compared to controls when quantified by real-time PCR; Figs. 3C, 4C).

Resveratrol has Neuroprotective Effects in NMDA–Induced Retinal Damage in Adult Zebrafish

As shown in Figure 5, the nerve fiber and ganglion cell layers of adult zebrafish retina were significantly thicker in the NMDA treatment group (Figs. 5A, 5B). Compared to the control retinas, resveratrol did not cause retinal damage and seemed to have neuroprotective effects in the NMDA treatment group with resveratrol. Consistently, NMDA did not alter the mRNA level of *SIRT1*, *SIRT3-5*, and *Fis1* genes in the adult zebrafish liver (Supplementary Figs. S1A–D, S1F). There was a small increase in *SIRT1* gene expression, and SIRT1 protein expression decreased in the retina following NMDA-induced retinal damage (Figs. 5C, 5E). However, *SIRT1* gene and protein expression increased after exposure to NMDA with resveratrol. Additionally, *OPA1* gene expression showed no change while the protein expression increased following NMDA-induced retinal damage (Figs. 5D, 5E). In the NMDA plus resveratrol treatment group, there was no change in OPA1 protein expression in the retina compared to the controls. Furthermore, *SIRT3-5* and *Fis1* gene expression significantly increased in NMDA-induced retinal damage in the adult zebrafish retina at 24 hours (Figs. 5F–I).

In addition, the antioxidant resveratrol improved mitochondrial DNA repair and did not increase mitCN in the normal retina (Figs. 6A, 6B). However, mitCN increased while there was no mitochondrial DNA damage following NMDA-induced retinal damage. In the NMDA treatment group with resveratrol, mitCN showed a slight suppression of upregulation when compared to the NMDA treatment group (Fig. 6B).

DISCUSSION

The zebrafish model has been used extensively to test the effect of different substances in the retina. We used this model to assess the effects of resveratrol in the zebrafish retina. To establish a basis for studying the functions of the sirtuins in zebrafish retina, we first analyzed the mRNA expression pattern of sirtuins (SIRT1-7). Our data showed that low and high concentrations of resveratrol increased SIRT1, mitochondrial sirtuins, and mitochondrial gene (*OPA1* and *Fis1*) expression at different time points in the retina. Particularly, the *SIRT4* gene and protein were highly expressed in the retina following resveratrol treatment. Moreover, our results demonstrated that resveratrol, an activator of SIRT1, offered protection against NMDA-induced retinal damage by potentially regulating mitochondrial sirtuins and mitochondrial genes in zebrafish retina.

Prior studies have found that *SIRTs* gene are expressed in zebrafish tissue (spleen, gills, brain, liver, among others).[29] Nevertheless, the significance of SIRTs expression in the zebrafish retina is unknown. We previously investigated SIRTs expression in vertebrates (rat, mouse, and human) in detail.[33] Now, we directed our focus to analyze the expression pattern of *SIRTs* gene expression in the zebrafish model. Our results demonstrated that all seven SIRTs were highly expressed in the retina when compared to the liver. This finding was consistent with the level of sirtuin mRNA expression in the retina, as it is one of the highest energy-consuming tissues in the body.[32,34] Moreover, we observed that mitochondrial sirtuin gene expression had a large proportion of genes in the SIRT's family. In previous studies, mitochondrial sirtuins regulated mitochondrial function and pathogenesis for age-related disorders.[35] Aging is a known risk factor for glaucoma. Therefore, mitochondrial sirtuins presented promising targets for studying this pathology.[35,36]

We found that OPA1, a mitochondrial dynamin-related protein, had a higher level of mRNA expression in retina when compared to the liver. However, Fis1 mRNA expression showed no difference between the two tissues. We postulated that OPA1 may have a role in RGC synaptic architecture[37] and have a high expression in RGCs.[38] Some studies have shown that OPA1 mutations cause optic atrophy in retinal ganglion

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 04/24/2024

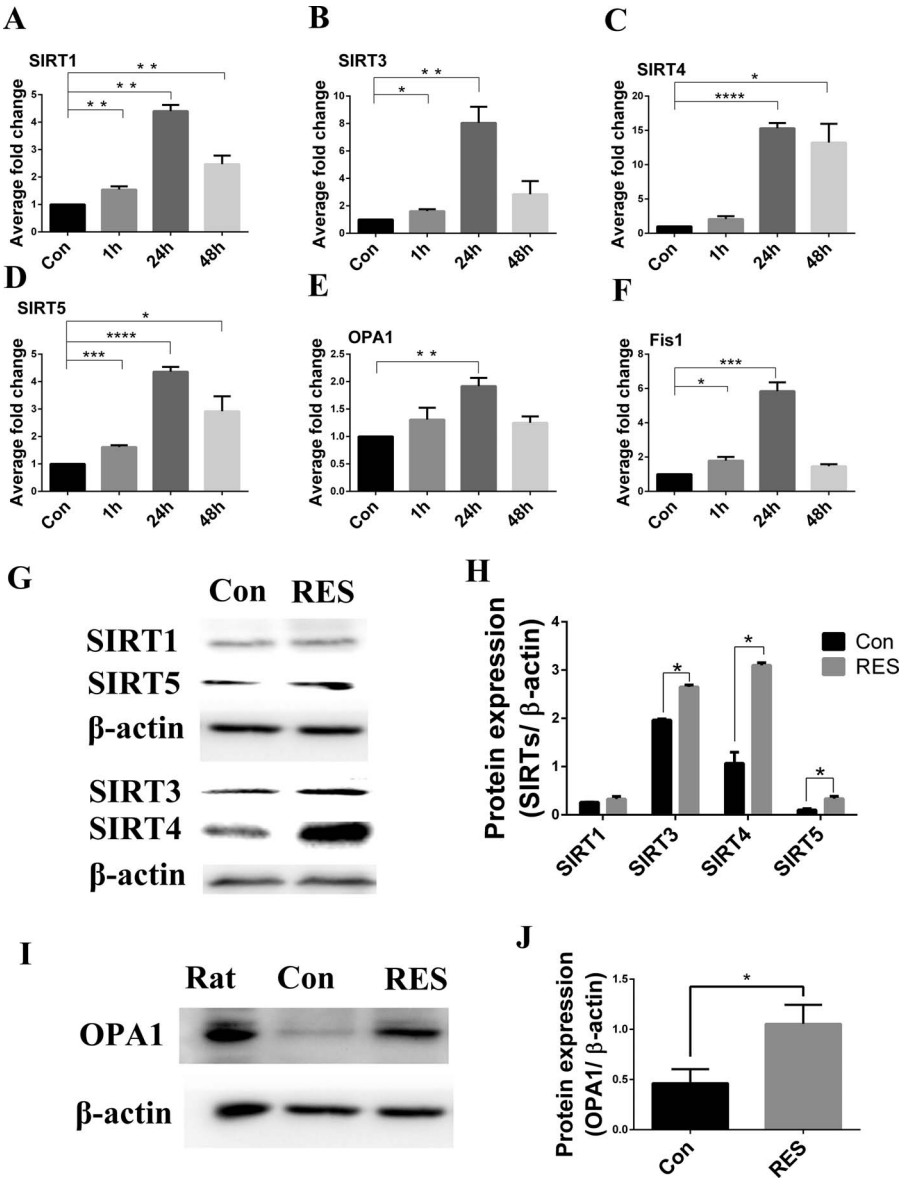

FIGURE 4. Effect of high concentration of resveratrol on SIRTs and mitochondrial gene (*OPA1* and *Fis1*) expression in adult zebrafish retina. (**A**–**F**) SIRTs, OPA1 and Fis1 expression in zebrafish retina after exposure to 50 mg/L resveratrol for 1, 24, and 48 hours. Graphic representation of average fold changes in (**A**) *SIRT1*, (**B**), *SIRT3*, (**C**) *SIRT4*, (**D**) *SIRT5*, (**E**) *OPA1*, and (**F**) *Fis1* gene expression by quantitative real-time PCR. *Error bars*: SEM; $n = 4$ for qPCR (unpaired *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ compared to control). (**G**, **H**) Representative Western blot showing the level of SIRT1, SIRT3, SIRT4, and SIRT5 protein expression after exposure to 50 mg/L resveratrol for 24 hours in the retina. The histograms in each Figure show the densitometric mean and SEM normalized to the corresponding level of the loading control protein β-actin (unpaired *t*-test, $^{*}P < 0.05$, $n = 4$). Con, control; RES, 50 mg/L resveratrol. (**I**, **J**) Representative Western blot showing the level of OPA1 protein expressions after exposure to 50 mg/L resveratrol for 24 hours in the retina. The histograms in each Figure show the densitometric mean and SEM normalized to the corresponding level of the loading control protein β-actin (unpaired *t*-test, $^{*}P < 0.05$, $n = 4$). Rat served as a positive control; Con, control; RES, 50 mg/L resveratrol.

cell pathophysiology,[21] and downregulation of OPA1 led to aggregation of the mitochondrial network in RGCs.[39]

When resveratrol was added to zebrafish's water, it was rapidly absorbed by the blood vessels in the gills and skin. The compounds diffused through systemic circulation and reached target tissue, which then produced a response. In this study, we found resveratrol treatment increased *SIRT1* gene and protein expression in the zebrafish retina. Previous work by other laboratories has established resveratrol as a potent activator of SIRT1.[10,40,41] Rats treated with resveratrol that were not stressed or impaired also showed an increase in SIRT1 protein levels.[42] In the literature, resveratrol has been shown to significantly increase aerobic capacity and improve mitochondrial function by activating SIRT1 and PGC-1α.[43] Studies have demonstrated that SIRT1 activation, which is considered to be the protective effect mediated by resveratrol, reduced brain edema and neuronal apoptosis.[41] We also provided observational support for the role of SIRT1 mediating a protective effect against NMDA-induced retinal edema in the zebrafish retina. Interestingly, resveratrol did not alter SIRT1 mRNA expression in the zebrafish liver. Some factors that should be considered are that resveratrol is not a direct activator of SIRT1, different tissue types can affect this modulation, and there are

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 04/24/2024

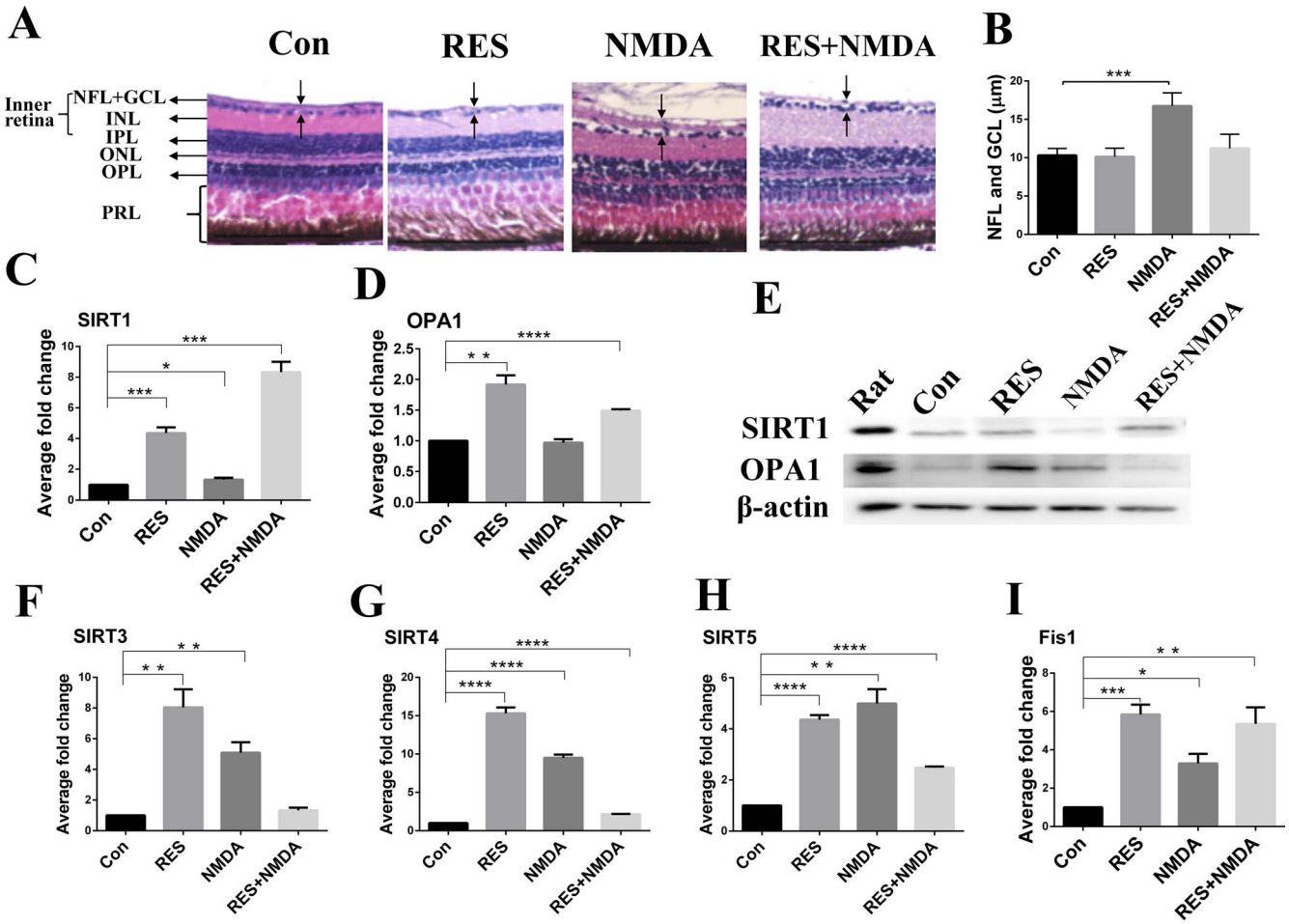

FIGURE 5. Effect of resveratrol on retinal SIRTs and mitochondrial gene expression after NMDA-induced retinal degeneration. (**A**) Comparison of retinal morphology of control and treatment groups in the peripheral retina. HE staining of retinal sections are shown. *Scale bar*: 100 μm. NFL, nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; PRL, photoreceptor layer. (**B**) Quantification of the nerve fiber and GCLs of adult zebrafish retina. *Error bars*: SEM; $n = 5$ for HE stained. (unpaired *t*-test, $^{***}P < 0.001$ compared to control) (**C**, **D**, **F**–**I**) *SIRTs*, *OPA1*, and *Fis1* genes expression in zebrafish retina after exposure to 50 mg/L resveratrol,100 μM NMDA and 100 μM NMDA with 50 mg/L resveratrol for 24 hours. Graphic representation of average fold changes in (**C**) *SIRT1*, (**D**) *OPA1*, (**F**) *SIRT3*, (**G**) *SIRT4*, (**H**) *SIRT5*, and (**I**) *Fis1* gene expression by qPCR. *Error bars*: SEM; $n = 4$ for qPCR. (unpaired *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ compared to control) (**E**) Representative Western blot showing the level of SIRT1 and OPA1 protein expression of control and treatment groups for the 24 -hour time point in the retina. Rat served as a positive control; Con, control; RES, 50 mg/L resveratrol; NMDA, 100 μM NMDA; NMDA+ RES, 100 μM NMDA with 50 mg/L resveratrol.

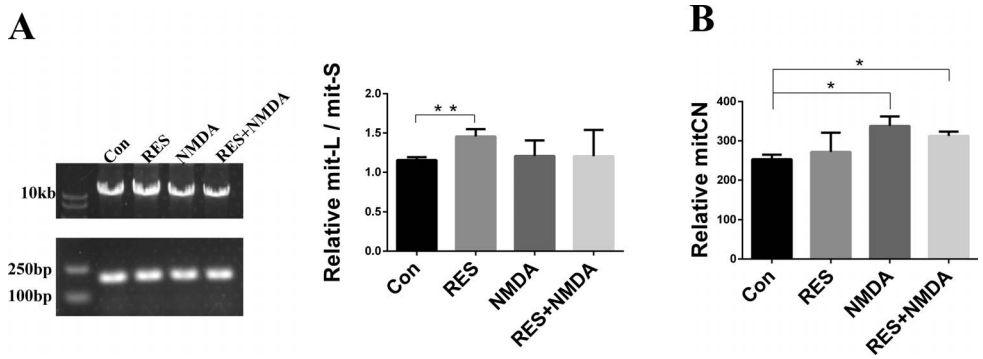

FIGURE 6. Resveratrol improved mitochondrial function in normal retina and damaged retina. (**A**) The *leftmost band* is the DNA marker. The representative gel of mitochondrial DNA damage assay shows that long mitochondrial segments and short mitochondrial segments had different band densities in the adult zebrafish retina after exposure to 50 mg/L resveratrol, 100 μM NMDA, and 100 μM NMDA with 50 mg/L resveratrol for 24 hours. The *graph* shows the mit-L/mit-S ratio obtained by optical densitometry analysis (mean + SEM, unpaired *t*-test, $^{**}P < 0.01$, $n = 3$). Mit-L, long mitochondrial segments; mit-S, short mitochondrial segments. (**B**) Comparison of retinal mitCN of control and treatment groups for 24 hours (unpaired *t*-test, $^{*}P < 0.05$, $n = 3$) Con, control; RES, 50 mg/L resveratrol; NMDA, 100 μM NMDA; NMDA+ RES, 100 μM NMDA with 50 mg/L resveratrol.

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 04/24/2024

differences in the distribution of resveratrol in different tissue types.[23,44]

SIRT3, SIRT4, and SIRT5, located in the mitochondria, control basic mitochondrial biology, including energy production and metabolism, apoptosis, and intracellular signaling.[45] We found that resveratrol increased mitochondrial sirtuin gene and protein expression in the zebrafish retina. Previous reports showed that resveratrol reduced oxidative stress under SIRT3 control in mitochondrial complex I deficiency.[46] In human vascular endothelial cells, resveratrol also maintained mitochondrial reactive oxygen species homeostasis through activation of the SIRT3 signaling pathway.[47] An interesting and novel finding of our study was that the effect of resveratrol on *SIRT4* gene expression increased 15-fold when compared to control, and protein expression highly increased in the zebrafish retina. Similar studies have suggested that resveratrol affected the antiaging process through a novel mechanism via the NAMPT-SIRT4-HTERT axis.[48] Additionally, SIRT4 inhibited mitochondrial glutamine metabolism to prevent DNA damage,[49,50] and regulated mitochondrial dynamics and mitophagy in sensitized cells to mitochondrial stress.[17] We also observed that resveratrol improved mitochondrial DNA repair in the normal zebrafish retina. However, the mechanisms of regulation remain poorly understood. Further investigation of the mechanisms of SIRT4 expression and its role in retinal diseases are necessary. Additionally, while SIRT5 is a mitochondrial sirtuin that regulates the urea cycle, it also has neuronal protective effects and a proapoptotic effect in cerebellar granule neurons.[18,51] Therefore, mitochondrial sirtuins might become a new target for glaucoma treatment.

Interestingly, we found that resveratrol increased *OPA1* gene and protein expression. Some studies have demonstrated that the *OPA1* gene was a major marker indicating normal tension glaucoma, and primary open angle glaucoma patients had low expression of *OPA1*.[52,53] One prior study found resveratrol altered expression of mitochondrial fusion and fission genes in replicative senescent yeast cells.[54] Therefore, we have provided observational support for resveratrol's protective effects within the retina by activating *OPA1*.We found OPA1 protein expression increased following NMDA-induced retinal damage in the zebrafish retina, as reported previously.[55] Some studies also showed that glutamate receptor activation triggered OPA1 release and had the potential for proteolytic processing of OPA1.[55] The *Fis1* gene also is positively regulated following NMDA treatment. Prior studies have shown that chronic low levels of hydrogen peroxide increased mtDNA copy number in isolated yeast mitochondria and that treatment of human cells with hydrogen peroxide resulted in a transient increase in mitCN and mitochondrial mass.[56,57] High concentrations of NMDA induced apoptosis in the rat retina[58] and low concentrations of NMDA resulted in ROS production in the zebrafish retina, revealing the positive regulation of mitDNA copy number and possibly an increase in mitochondrial mass. Therefore, Opa1, as a fusion protein, and Fis1 increased expression patterns in the retinal damage model. A similar result was observed in that homocysteine-induced ganglion cell loss involved the dysregulation of mitochondrial dynamics, which increased *Opa1* and *Fis1* gene and protein expression.[59] Further investigation is needed to confirm that resveratrol offers protection against glaucoma by regulating mitochondrial dynamics.

Prior studies have established a model of NMDA-induced damage in the rat retina,[58] and a series of neuroprotective substances have been found using this model.[60–62] We first initiated NMDA-induced retinal damage in the zebrafish retina. NMDA, as a specific agonist of the NMDA receptor, is an excitotoxin used frequently in neuroscience research. In our study, NMDA did not alter the mRNA level of sirtuins in the adult zebrafish liver. We also observed that the nerve fiber and ganglion cell layers were thicker (edema) and the number of RGCs did not change (data no shown) after NMDA treatment. In the pathogenesis of glaucoma, RGC apoptosis also occurs.[63] Further investigation is needed urgently to establish a model of NMDA-induced glaucoma in zebrafish.

Due to the pharmokinetics and instability of resveratrol, the effect of resveratrol on SIRTs and mitochondrial gene expression increased first, but then was followed by a decrease. Therefore, we chose sirtuins and mitochondrial genes that were highly expressed after a 24-hour exposure to high concentrations of resveratrol to protect against NMDA-induced retinal damage in zebrafish. Interestingly, there was a small increase of SIRT1 gene expression, but there was a decrease in SIRT1 protein expression after NMDA treatment in the zebrafish retina. This suggested that the posttranscription mechanisms of SIRT1 might transmit RNA-binding proteins or noncoding small RNAs.[33,64] Previous studies also demonstrated that the protein expression of SIRT1 decreased with age and injury in the rat retina.[33] In the NMDA plus resveratrol treatment group, SIRT1 expression was significantly increased in the zebrafish retina when compared to mitochondrial sirtuins and mitochondrial genes (*OPA1* and *Fis1*). Previous studies also have shown that resveratrol or overexpression of SIRT1 elicited inhibitory effects on NMDA-induced excitotoxicity and regulated mitochondrial fission/fusion and biogenesis in neurotoxicity.[65,66] Additionally, prior studies revealed that resveratrol suppressed glutamatergic neurotransmission via the NMDA receptor in vivo and inhibited calcium channels.[67,68] Hence, mitochondrial sirtuins, OPA1 mRNA, and mitCN showed suppressed upregulation in the treatment group receiving NMDA with resveratrol, possibly through the inhibition of glutamatergic neurotransmission and/or calcium channels via NMDA receptors. Further studies will be necessary to confirm the connection between sirtuin expression and mitochondrial genes in mediating the protection from retinal damage. All in all, our study provided evidence that resveratrol was protective against retinal neuropathies, and it is possible that resveratrol can prevent or treat glaucoma in the future.

Conclusions

To our knowledge, our study represents the first investigation of the expression of sirtuins in adult zebrafish retina, which will help elucidate the role of sirtuins in the regulation of zebrafish retinal metabolism. Moreover, we showed the effect of different concentrations of resveratrol at different time points on SIRTs (SIRT1, 3, 4, and 5) and mitochondrial genes (*OPA1* and *Fis1*) in the adult zebrafish retina. Finally, we also found that resveratrol, as a nutraceutical compound, impeded the progression of NMDA-induced retinal damage by potentially mediating mitochondrial sirtuins and *OPA1* genes and suggested that zebrafish is a good model for future studies of retinal degeneration and for screening compounds for glaucoma treatment.

Acknowledgments

Supported by the National Natural Science Foundation of China (81271425, 81260148, 31400988, 81160144, and 31171044) and Natural Science Foundation of Jiangxi (20151BBG70243, 20122BCB23007).

Disclosure: **W. Sheng**, None; **Y. Lu**, None; **F. Mei**, None; **N. Wang**, None; **Z.-Z. Liu**, None; **Y.-Y. Han**, None; **H.-T. Wang**, None; **S. Zou**, None; **H. Xu**, None; **X. Zhang**, None

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 04/24/2024

Investigative Ophthalmology & Visual Science

References

1. Mozaffarieh M, Grieshaber MC, Flammer J. Oxygen and blood flow: players in the pathogenesis of glaucoma. *Mol Vis*. 2008; 14:224–233.
2. Yang X-J, Ge J, Zhuo Y-H. Role of mitochondria in the pathogenesis and treatment of glaucoma. *Chin Med J (Engl)*. 2013;126:4358–4365.
3. Malhotra A, Bath S, Elbarbry F. An organ system approach to explore the antioxidative, anti-inflammatory, and cytoprotective actions of resveratrol. *Oxid Med Cell Longev*. 2015;2015: 803971.
4. Das DK, Mukherjee S, Ray D. Resveratrol and red wine, healthy heart and longevity. *Heart Fail Rev*. 2010;15:467–477.
5. Yar AS, Menevse S, Alp E, Helvacioglu F, Take G. The effects of resveratrol on cyclooxygenase-1 and cyclooxygenase-2 mRNA and protein levels in diabetic rat kidneys. *Mol Biol Rep*. 2010; 37:2323–2331.
6. Marques FZ, Markus MA, Morris BJ. Resveratrol: cellular actions of a potent natural chemical that confers a diversity of health benefits. *Int J Biochem Cell Biol*. 2009;41:2125–2128.
7. Szkudelska K, Szkudelski T. Resveratrol, obesity and diabetes. *Eur J Pharmacol*. 2010;635:1–8.
8. Patel KR, Brown VA, Jones DJL, et al. Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. *Cancer Res*. 2010;70:7392–7399.
9. Hsieh T, Wu JM. Resveratrol: biological and pharmaceutical properties as anticancer molecule. *BioFactors*. 2010;36:360–369.
10. Yun JM, Chien A, Jialal I, Devaraj S. Resveratrol up-regulates SIRT1 and inhibits cellular oxidative stress in the diabetic milieu: mechanistic insights. *J Nutr Biochem*. 2012;23:699–705.
11. Liu X-Q, Wu B-J, Pan WHT, et al. Resveratrol mitigates rat retinal ischemic injury: the roles of matrix metalloproteinase-9, inducible nitric oxide, and heme oxygenase-1. *J Ocul Pharmacol Ther*. 2013;29:33–40.
12. Huang W, Li G, Qiu J, Gonzalez P, Challa P. Protective effects of resveratrol in experimental retinal detachment. *PLoS One*. 2013;8:e75735.
13. Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. *Biochem J*. 2007;404:1–13.
14. Kim SH, Lu HF, Alano CC. Neuronal sirt3 protects against excitotoxic injury in mouse cortical neuron culture. *PLoS One*. 2011;6:e14731.
15. Tseng AHH, Shieh SS, Wang DL. SIRT3 deacetylates FOXO3 to protect mitochondria against oxidative damage. *Free Radic Biol Med*. 2013;63:222–234.
16. Bell EL, Guarente L. The SirT3 divining rod points to oxidative stress. *Mol Cell*. 2011;42:561–568.
17. Lang A, Anand R, Altinoluk-Hambüchen S, et al. SIRT4 interacts with OPA1 and regulates mitochondrial quality control and mitophagy. *Aging (Albany NY)*. 2017;9:2160–2186.
18. Pfister JA, Ma C, Morrison BE, D'Mello SR. Opposing effects of sirtuins on neuronal survival: SIRT1-mediated neuroprotection is independent of its deacetylase activity. *PLoS One*. 2008;3:e4090.
19. Samant SA, Zhang HJ, Hong Z, et al. SIRT3 Deacetylates and Activates OPA1 to regulate mitochondrial dynamics during stress. *Mol Cell Biol*. 2014;34:807–819.
20. Yoon Y, Krueger EW, Oswald BJ, McNiven MA. The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamin-like protein DLP1. *Mol Cell Biol*. 2003;23:5409–5420.
21. Delettre C, Lenaers G, Griffoin JM, et al. Nuclear gene OPA1, encoding a mitochondrial dynamin-related protein, is mutated in dominant optic atrophy. *Nat Genet*. 2000;26:207–210.
22. Zon LI, Peterson RT. In vivo drug discovery in the zebrafish. *Nat Rev Drug Discov*. 2005;4:35–44.
23. Schirmer H, Pereira TCB, Rico EP, et al. Modulatory effect of resveratrol on SIRT1, SIRT3, SIRT4, PGC1α and NAMPT gene expression profiles in wild-type adult zebrafish liver. *Mol Biol Rep*. 2012;39:3281–3289.
24. Chhetri J, Jacobson G, Gueven N. Zebrafish-on the move towards ophthalmological research. *Eye*. 2014;28:367–380.
25. Barbazuk WB, Korf I, Kadavi C, et al. The syntenic relationship of the zebrafish and human genomes. *Genome Res*. 2000;10:1351–1358.
26. Parng C, Seng WL, Semino C, McGrath P. Zebrafish: a preclinical model for drug screening. *Assay Drug Dev Technol*. 2002;1:41–48.
27. Santoriello C, Zon LI. Hooked! modeling human disease in zebrafish. *J Clin Invest*. 2012;122:2337–2343.
28. Cao R, Jensen LDE, Söll I, Hauptmann G, Cao Y. Hypoxia-induced retinal angiogenesis in zebrafish as a model to study retinopathy. *PLoS One*. 2008;3:e2748.
29. Pereira TCB, Rico EP, Rosemberg DB, et al. Zebrafish as a model organism to evaluate drugs potentially able to modulate sirtuin expression. *Zebrafish*. 2011;8:9–16.
30. Hunter S, Jung D, Di Giulio R, Meyer J. The QPCR assay for analysis of mitochondrial DNA damage, repair, and relative copy number. *Methods*. 2010;51:444–451.
31. Foote K, Reinhold J, Yu EPK, et al. Restoring mitochondrial DNA copy number preserves mitochondrial function and delays vascular aging in mice [published online ahead of print May 9, 2018]. *Aging Cell*. doi:10.1111/acel.12773.
32. Ban N, Ozawa Y, Inaba T, et al. Light-dark condition regulates sirtuin mRNA levels in the retina. *Exp Gerontol*. 2013;48: 1212–1217.
33. Luo H, Zhou M, Ji K, et al. Expression of sirtuins in the retinal neurons of mice, rats, and humans. *Front Aging Neurosci*. 2017;9:366.
34. Niven JE, Laughlin SB. Energy limitation as a selective pressure on the evolution of sensory systems. *J Exp Biol*. 2008;211:1792–1804.
35. van de Ven RAH, Santos D, Haigis MC. Mitochondrial sirtuins and molecular mechanisms of aging. *Trends Mol Med*. 2017; 23:320–331.
36. Shih J, Donmez G. Mitochondrial sirtuins as therapeutic targets for age-related disorders. *Genes Cancer*. 2013;4:91–96.
37. Williams PA, Piechota M, Von Ruhland C, Taylor E, Morgan JE, Votruba M. Opa1 is essential for retinal ganglion cell synaptic architecture and connectivity. *Brain*. 2012;135:493–505.
38. Ju W-K, Misaka T, Kushnareva Y, et al. OPA1 expression in the normal rat retina and optic nerve. *J Comp Neurol*. 2005;488: 1–10.
39. Kamei S, Chen-Kuo-Chang M, Cazevieille C, et al. Expression of the Opa1 mitochondrial protein in retinal ganglion cells: its downregulation causes aggregation of the mitochondrial network. *Invest Ophthalmol Vis Sci*. 2005;46: 4288–4294.
40. Lagouge M, Argmann C, Gerhart-Hines Z, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. *Cell*. 2006;127: 1109–1122.
41. Qian C, Jin J, Chen J, et al. SIRT1 activation by resveratrol reduces brain edema and neuronal apoptosis in an experimental rat subarachnoid hemorrhage model. *Mol Med Rep*. 2017;16:9627–9635.
42. Barger JL, Kayo T, Pugh TD, Prolla TA, Weindruch R. Short-term consumption of a resveratrol-containing nutraceutical mixture mimics gene expression of long-term caloric restriction in mouse heart. *Exp Gerontol*. 2008;43:859–866.

Downloaded from iovs.arvojournals.org on 04/24/2024

43. Price NL, Gomes AP, Ling AJY, et al. SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. *Cell Metab*. 2012;15:675–690.
44. Beher D, Wu J, Cumine S, et al. Resveratrol is not a direct activator of sirt1 enzyme activity. *Chem Biol Drug Des*. 2009; 74:619–624.
45. Verdin E, Hirschey MD, Finley LWS, Haigis MC. Sirtuin regulation of mitochondria: Energy production, apoptosis, and signaling. *Trends Biochem Sci*. 2010;35:669–675.
46. Mathieu L, Costa AL, Le Bachelier C, et al. Resveratrol attenuates oxidative stress in mitochondrial Complex I deficiency: involvement of SIRT3. *Free Radic Biol Med*. 2016;96:190–198.
47. Zhou X, Chen M, Zeng X, et al. Resveratrol regulates mitochondrial reactive oxygen species homeostasis through Sirt3 signaling pathway in human vascular endothelial cells. *Cell Death Dis*. 2014;5:e1576.
48. Huang P, Riordan SM, Heruth DP, Grigoryev DN, Zhang LQ, Ye SQ. A critical role of nicotinamide phosphoribosyltransferase in human telomerase reverse transcriptase induction by resveratrol in aortic smooth muscle cells. *Oncotarget*. 2015; 6:10812–10824.
49. Haigis MC, Mostoslavsky R, Haigis KM, et al. SIRT4 Inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic β cells. *Cell*. 2006;126:941–954.
50. Jeong SM, Xiao C, Finley LWS, et al. SIRT4 has tumor-suppressive activity and regulates the cellular metabolic response to DNA damage by inhibiting mitochondrial glutamine metabolism. *Cancer Cell*. 2013;23:450–463.
51. Nakagawa T, Guarente L. Urea cycle regulation by mitochondrial sirtuin, SIRT5. *Aging (Albany NY)*. 2009;1:578–581.
52. Aung T, Ocaka L, Ebenezer ND, et al. A major marker for normal tension glaucoma: association with polymorphisms in the OPA1 gene. *Hum Genet*. 2002;110:52–56.
53. Bosley TM, Hellani A, Spaeth GL, et al. Down-regulation of OPA1 in patients with primary open angle glaucoma. *Mol Vis*. 2011;17:1074–1079.
54. Wang IH, Chen HY, Wang YH, Chang KW, Chen YC, Chang CR. Resveratrol modulates mitochondria dynamics in replicative senescent yeast cells. *PLoS One*. 2014;9:e104345.
55. Ju W-K, Lindsey JD, Angert M, Patel A, Weinreb RN. Glutamate receptor activation triggers OPA1 release and induces apoptotic cell death in ischemic rat retina. *Mol Vis*. 2008; 14:2629–2638.
56. Hori A, Yoshida M, Shibata T, Ling F. Reactive oxygen species regulate DNA copy number in isolated yeast mitochondria by triggering recombination-mediated replication. *Nucleic Acids Res*. 2009;37:749–761.
57. Wei YH, Lee CF, Lee HC, et al. Increases of mitochondrial mass and mitochondrial genome in association with enhanced oxidative stress in human cells harboring 4,977 BP-deleted mitochondrial DNA. *Ann N Y Acad Sci*. 2001;928:97–112.
58. Lam TT, Abler AS, Kwong JMK, Tso MOM. N-methyl-D-aspartate (NMDA)-induced apoptosis in rat retina. *Invest Ophthalmol Vis Sci*. 1999;40:2391–2397.
59. Ganapathy PS, Perry RL, Tawfik A, et al. Homocysteine-mediated modulation of mitochondrial dynamics in retinal ganglion cells. *Invest Ophthalmol Vis Sci*. 2011;52:5551–5558.
60. Lambuk L, Jafri AJA, Arfuzir NNN, et al. Neuroprotective effect of magnesium acetyltaurate against NMDA-induced excitotoxicity in rat retina. *Neurotox Res*. 2017;31:31–45.
61. Sakamoto K, Okuwaki T, Ushikubo H, Mori A, Nakahara T, Ishii K. Activation inhibitors of nuclear factor kappa B protect neurons against the NMDA-induced damage in the rat retina. *J Pharmacol Sci*. 2017;135:72–80.
62. Maekawa S, Sato K, Fujita K, et al. The neuroprotective effect of hesperidin in NMDA-induced retinal injury acts by suppressing oxidative stress and excessive calpain activation. *Sci Rep*. 2017;7:6885.
63. Quigley HA, Nickells RW, Kerrigan LA, Pease ME, Thibault DJ, Zack DJ. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. *Invest Ophthalmol Vis Sci*. 1995;36:774–786.
64. Sidorova-Darmos E, Wither RG, Shulyakova N, et al. Differential expression of sirtuin family members in the developing, adult, and aged rat brain. *Front Aging Neurosci*. 2014;6:333.
65. Yang X, Si P, Qin H, Yin L, Yan LJ, Zhang C. The neuroprotective effects of SIRT1 on NMDA-induced excitotoxicity. *Oxid Med Cell Longev.* 2017;2017:2823454.
66. Peng K, Tao Y, Zhang J, et al. Resveratrol regulates mitochondrial biogenesis and fission/fusion to attenuate rotenone-induced neurotoxicity. *Oxid Med Cell Longev.* 2016;2016:6705621.
67. Takehana S, Kubota Y, Uotsu N, et al. The dietary constituent resveratrol suppresses nociceptive neurotransmission via the NMDA receptor. *Mol Pain*. 2017;13:1744806917697010.
68. Pan X, Chen J, Wang W, et al. Resveratrol-induced antinociception is involved in calcium channels and calcium/caffeine-sensitive pools. *Oncotarget*. 2017;8:9399–9409.

Investigative Ophthalmology & Visual Science

Downloaded from iovs.arvojournals.org on 04/24/2024