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

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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 sirtuins (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 cosin 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

G laucoma, 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 Polygonum 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
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. Additionally, approximately 70% to 80% of zebrafish genes share homology with the human genome. Zebrafish bioassays also are cheaper and faster than mouse assays, and can be used to assess toxicity responses, teratogenic effects, and LC(50). 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). For example, the zebrafish mutants (lamb and pax2) reveal the potential of aminoglycosides in the treatment of human coloboma. Therefore, zebrafish models have been used to test oral angiogenesis inhibitors to rescue neovascularization of the retina. Zebras are 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 damage in adult zebrafish? Third, what is the effect of resveratrol in the zebrafish retina. First, are SIRTs and mitochondrial target genes expressed in wild-type adult zebrafish retina? Second, what is the effect of resveratrol on mitochondrial target (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. 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.

**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 THAImScript RT kit (Tiangen, Beijing, China), which is a synthesis system for RT-PCR, according to the manufacturer’s instructions. PCR cycles were 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

**TABLE 1.** PCR Primers Used in the Study

<table>
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<tr>
<th>Genome</th>
<th>Primers (5’ to 3’)</th>
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<td>SIRT1</td>
<td>F: CAAGGAAAATCTACCCCCGAGACAGT</td>
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<tr>
<td></td>
<td>R: CAGTGCTGTAGATTTCTTGCTGAGT</td>
</tr>
<tr>
<td>SIRT2</td>
<td>F: ATCCCAAGATTTCCCTCCTCC</td>
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<tr>
<td></td>
<td>R: AGGCCCTAGAATGCTTGG</td>
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<tr>
<td>SIRT3</td>
<td>F: CCTTGTATATCCCTCCGACAAC</td>
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<tr>
<td></td>
<td>R: CATGCTTCACAGTAAGAGAAC</td>
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<td>SIRT4</td>
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<td></td>
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<tr>
<td></td>
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<tr>
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<td></td>
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<tr>
<td>β-actin</td>
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<td></td>
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<tr>
<td>Mitochondrial target (198 bp)</td>
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<tr>
<td></td>
<td>R: CACTGATTTAGGGGAGACAGT</td>
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RES, 5 mg/L resveratrol

OPA1 BD 612606 Mouse mAb 1:1000 80–100

SIRT5 Abcam 78982 Rabbit polyclonal 1:1000 34

SIRT4 Abcam 10140 Goat polyclonal 1:1000 36

SIRT3 Abcam 86671 Rabbit mAb 1:1000 36.6

SIRT1 Sigma s5447 Rabbit mAb 1:1000 120

Antibody Source Catalog No. Type of Ab Dilution MW, kD

Table 2. Primary Antibodies Used in the Study

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<th>Source</th>
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<th>Type of Ab</th>
<th>Dilution</th>
<th>MW, kD</th>
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<td>86671</td>
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<td>10140</td>
<td>Goat polyclonal</td>
<td>1:1000</td>
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<tr>
<td>SIRT5</td>
<td>Abcam</td>
<td>78982</td>
<td>Rabbit polyclonal</td>
<td>1:1000</td>
<td>34</td>
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<tr>
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<td>612606</td>
<td>Mouse mAb</td>
<td>1:1000</td>
<td>80–100</td>
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<td>β-Actin</td>
<td>TRANS</td>
<td>HC201</td>
<td>Mouse mAb</td>
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<td>42</td>
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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). 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 adult zebrafish mitochondrial genomic DNA extraction kit (Sangon Biotech). Primer sequences for the mitochondrial segment (198 bp) and nuclear segment (233 bp) were as previously described (Table 1).
MitCN was calculated relative to nuclear DNA using the following equations:31

\[ \Delta C_T = \text{mitochondrial } C_T - \text{nuclear } C_T \]  

(1)

Relative mitochondrial DNA content = \(2^{2^{-\Delta C_T}}\)  

(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, 4I). High concentration of resveratrol significantly altered SIRTs and mitochondrial gene expression when compared to the low concentration. The effect of resveratrol on SIRT4 gene

\[
\Delta C_T = \text{mitochondrial } C_T - \text{nuclear } C_T
\]

(1)

Relative mitochondrial DNA content = \(2^{2^{-\Delta C_T}}\)

(2)
Mitochondrial Genes in Adult Zebrafish Retinas

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 genes are expressed in zebrafish tissue (spleen, gills, brain, liver, among others). 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. 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 zebrafish retina.

We found that OPA1, a mitochondrial dynamin-related protein, had a higher level of mRNA expression in 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. 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. Aging is a known risk factor for glaucoma. Therefore, mitochondrial sirtuins presented promising targets for studying this pathology.

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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).)
cell pathophysiology, and downregulation of OPA1 led to aggregation of the mitochondrial network in RGCs. 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. Rats treated with resveratrol that were not stressed or impaired also showed an increase in SIRT1 protein levels. In the literature, resveratrol has been shown to significantly increase aerobic capacity and improve mitochondrial function by activating SIRT1 and PGC-1α. Studies have demonstrated that SIRT1 activation, which is considered to be the protective effect mediated by resveratrol, reduced brain edema and neuronal apoptosis. 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

**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.
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) SIRT1, OPA1, and Fis1 gene 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.
we have provided observational support for resveratrol's effects in the retina.

Prior studies have established a model of NMDA-induced retinal degeneration in the 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.

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.

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#### References


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