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Resveratrol ameliorates disorders of mitochondrial biogenesis and dynamics in a rat chronic ocular hypertension model

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ABSTRACT

Aims: To explore the roles of mitochondrial biogenesis and dynamics in both RGC-5 cells apoptosis and rat retinal damage induced by elevated pressure and their involvement in resveratrol (RSV)-induced cell protection.
Materials and methods: The chronic ocular hypertension (COH) model was established in rats by injecting superparamagnetic iron oxide into anterior chamber. The RGC-5 cells were incubated under ambient and elevated pressure (70 mm Hg) respectively. The intraocular pressure (IOP) was measured using a handheld Tonolab tonometer; mitochondrial dysfunction was analyzed by membrane potential (MMP) depolarization, reactive oxygen species (ROS) level and transmission electron microscope (TEM) detection. Annexin V/PI staining and the terminal deoxynucleotidy transferase dUTP nick end labeling (TUNEL) staining assay were performed for apoptosis detection. Hematoxylin–Eosin staining was performed for retinal morphology detection. The expression of mitochondrial biogenesis and dynamics relating proteins were analyzed by western blot.
Key findings: The retinal morphology and mitochondrial function deteriorated in chronic ocular hypertension (COH) rats. The cells showed apoptosis and mitochondrial dysfunction under elevated pressure (70 mm Hg) incubation. Upregulating AMPK, NRF-1, Tfam, mfn-2, OPA1 expression with RSV-treatment could decrease the cell apoptosis, mitochondrial membrane potential depolarization, ROS generation both in in vitro and in vivo experiments, and normalized the retinal morphology in vivo.
Significance: Both in vitro and in vivo experiments demonstrated that activated AMPK/PGC-1α signaling pathway and improved expression of proteins were related to mitochondrial dynamics could be the possible mechanism underlying in the RSV's mitochondrial protection.

1. Introduction

Glaucoma is a complex degenerative optic neuropathy with loss of retinal ganglion cells (RGCs) [1]. RGCs death leads to gradual vision loss and ultimately blindness that is irreversible. Elevated intraocular pressure (IOP) is the major risk factor for the injury of RGCs in glaucoma and several mechanisms were proposed to contribute to the loss of RGCs, including neurotrophic factor deprivation, the intrinsic and extrinsic apoptosis pathways, excitotoxic damage and oxidative stress [2, 3]. Among these factors, mitochondrial dysfunction has been proved to play an important role in RGCs death as the neurons that selectively die in glaucoma demands high energy demands and are particularly dependent on mitochondrial function [4–7].

Mitochondrial biogenesis and dynamics (fission and fusion) helps to maintain the distribution of mitochondria and also is an important component of mitochondrial health and cellular health [8–10]: (1) Peroxisome proliferator-activated receptor-c coactivator a (PGC-1α) is the master regulator of mitochondrial biogenesis through binding of nuclear respiratory factors (NRF-1/2), which then activates the mitochondrial transcription factor A (TFAM), leading to replication of mtDNA [11, 12]. One major upstream regulator of PGC-1α is AMP-activated protein kinase (AMPK) and AMPK/PGC-1α represents the most important signaling pathway in mitochondrial biogenesis [13]. Overexpression or activated PGC-1α could increase mitochondrial mass and mitochondrial function in cell and animal models of mitochondrial disease [12, 14]; (2) Mitochondrial dynamics, including mitochondria fusion and fission, are regulated by a family of dynamin related guanosine triphosphatase (GTPases) and plays a key role in controlling mitochondrial shape, homeostasis and quality. Dysfunction in mitochondrial fission and fusion can result in various diseases, such as Parkinson's disease, Alzheimer's disease, and age-related macular degeneration [15, 16].

Resveratrol (3,5,41-trihydroxy-trans-stilbene, RSV) is a natural polyphenolic compound that is extracted from grapes, nuts and

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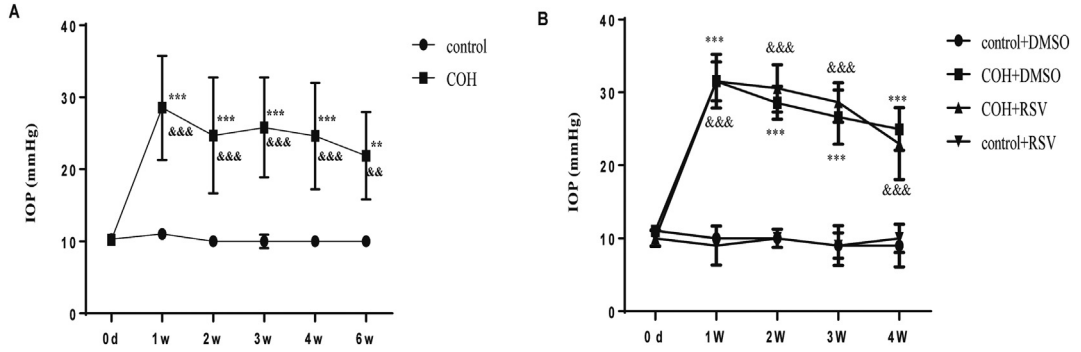

Fig. 1. Changes of IOP in eyes injected with micro-magnetic beads only and in eyes injected with micro-magnetic beads and treated with RSV for 4 weeks. (A) Difference of IOP between micro-magnetic beads injected eyes and control group for 6 weeks. **p < 0.01 and ***p < 0.001 vs. pre-operation; $^{\&\&}$p < 0.01, $^{\&\&\&}$p < 0.001 vs. control at the same time point. (B) Difference of IOP between RSV-treated and control group. ***p < 0.001 vs. pre-operation; $^{\&\&\&}$p < 0.001 vs. control + DMSO at the same time point.

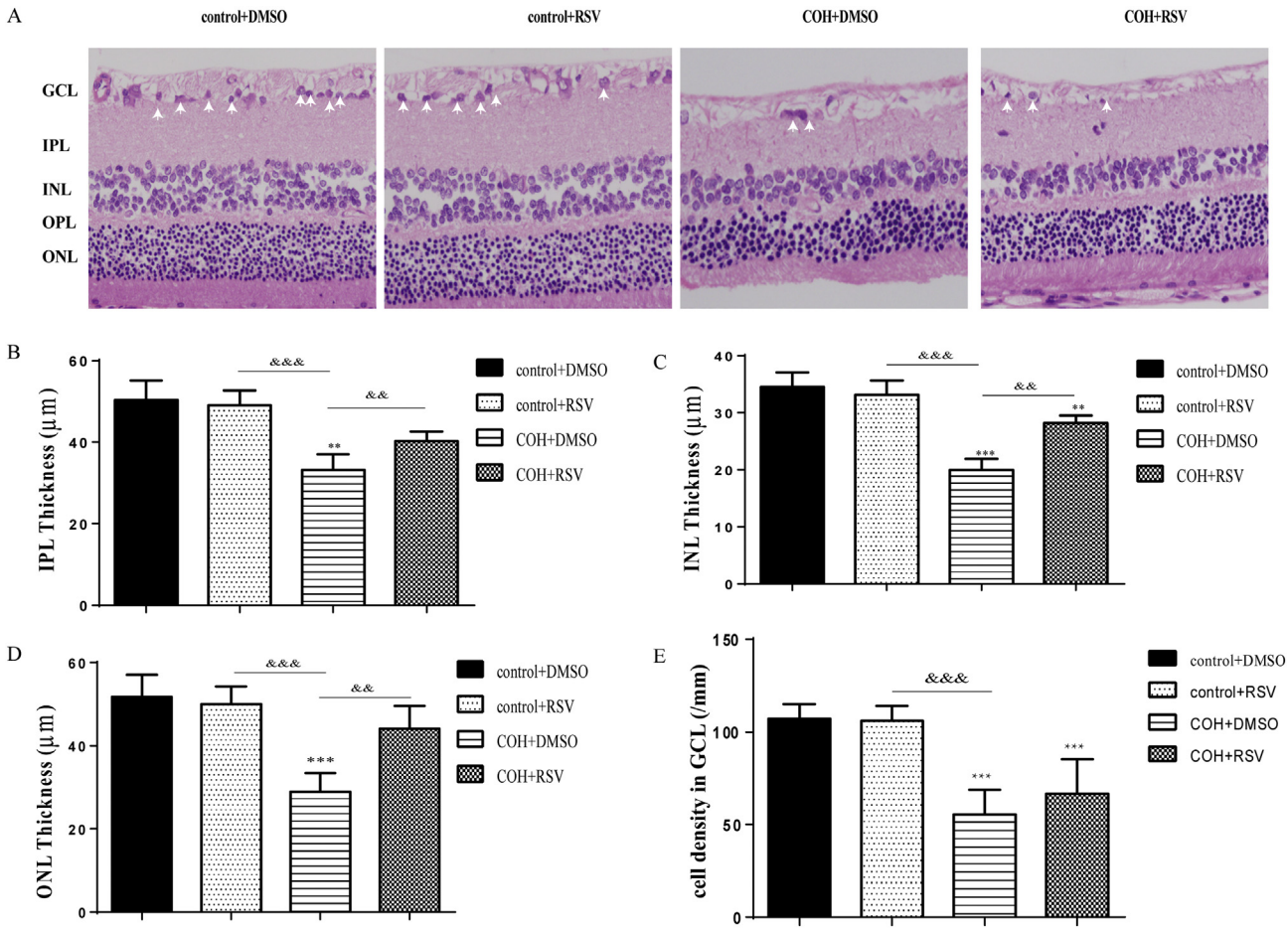

Fig. 2. Changes of retinal morphology in RSV-treated and no RSV-treated groups for 4 weeks. (A) Hematoxylin–Eosin staining of retinal slices at 4 weeks post-operatively. (B–E) Statistical analysis of the thickness of IPL, INL, ONL and cell density in GCL. **p < 0.01 and ***p < 0.001 vs. control + DMSO; $^{\&\&}$p < 0.01, $^{\&\&\&}$p < 0.001 vs. COH + DMSO.

pomegranates. In recent years, it is well known for its antioxidant and antitumorigenic properties, and neuroprotective effects, extending the lifespan [17–19]. In prior studies, RSV has been proved to protect human ARPE-19 cells from the oxidative stress damage via increased mitochondrial bioenergetics and protect against laser-induced CNV in animals [20]. In glaucoma, RSV could significantly delay the loss of RGCs in an experimental model [21, 22].

Despite mitochondrial dysfunction and protective effects of RSV on RGCs, the mechanisms underlying the protective effects of RSV on mitochondrial dysfunction induced by elevated pressure is not fully understand. Hence, in present study the protective effects of RSV against retinal damage and mitochondrial dysfunction in a glaucoma model were examined. Moreover, we explored the role of mitochondrial biogenesis and dynamics in RGC-5 cells apoptosis induced by elevated pressure and their involvement in RSV-induced cell protection.