High-Density Aromatic Peptide Protects Retinal Ganglion Cells and the Optic Nerve in Aging DBA/2J Mice

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INTRODUCTION

Mitochondrial dysfunction plays a critical role in the degeneration of retinal ganglion cells (RGCs) and their axons in neurodegenerative conditions such as glaucoma. Elevated intraocular pressure (IOP) induces mitochondrial stress, which leads to reduced ATP production, increased reactive oxygen species (ROS), and mitophagy. In glaucoma models, mitochondria show reduced numbers and altered morphology in affected axons, which contributes to RGC death. We hypothesize that by targeting mitochondrial function therapeutically, RGCs and the optic nerves could be protected from glaucomatous damage.

We have identified High-Density Aromatic Peptide 2 (HDAP2), a novel peptide designed to stabilize the mitochondrial membrane potential, enhance bioenergetics, and reduce cellular oxidative stress. HDAP2 binds to cardiolipin in mitochondrial membranes and optimizes proton trapping to mitigate stress-induced mitochondrial dysfunction. In vitro, HDAP2 improves the mitochondrial membrane potential, decreases oxidative stress, and prevents cell death under serum-free conditions.

This study evaluates the impact of HDAP2 on mitochondrial morphology, optic nerve preservation, and retinal ganglion cell survival in DBA/2J mice. HDAP2 is non-toxic, crosses the blood-retina barrier, and circumvents safety concerns associated with genetic therapies. These findings support HDAP2 as a promising mitochondria-targeted therapy for neuroprotection in glaucoma and other optic neuropathies.

METHODS

Animals and Treatments: We studied adult DBA/2J mice (3, 10, and 12 months old) obtained from The Jackson Laboratory (Bar Harbor, ME) and housed at York College, CUNY. Mice were tagged with RapID ear tags (RapID Lab, Inc.) to enable individual tracking of intraocular pressure (IOP), which was measured monthly using a rebound tonometer (Tonolab, iCare Finland Oy) under general anesthesia (Isoflurane, 2.5 ml/l O_2). HDAP2-treated animals received intraperitoneal injections (3 mg/kg) every other day starting at 4 months of age. All procedures were approved by the York College IACUC and followed the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Tissue Fixation and Processing: Mice were euthanized with ketamine (300 mg/kg) and xylazine (60 mg/kg), and their eyes were fixed in either 4% paraformaldehyde (for retinal RGC distribution and optic nerve structure) or a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (for TEM analysis). RGCs were labeled using standard immunocytochemistry. Optic nerves were embedded in Spurr's resin and sectioned with a Leica Ultracut microtome.

Microscopy: Retinas were imaged using a Zeiss LSM 900 Airyscan 2 confocal microscope with a 10x objective. Multiple tiles were acquired and stitched into high-resolution montages using ZEN Blue software. RGCs were counted in ImageJ with the "Analyze Particles" plugin. Optic nerve morphology was evaluated in semithin sections stained with toluidine blue and in 60 nm ultrathin sections mounted on copper grids and imaged with a JEOL JEM-1200EX TEM.

Statistical Analysis: GraphPad Prism was used to assess the effects of treatment and pressure on RGC counts, axonal structure, and mitochondrial morphology using ANOVA and t-tests.

RESULTS

1. IOPs of HDAP2 and Untreated groups were not significantly different.

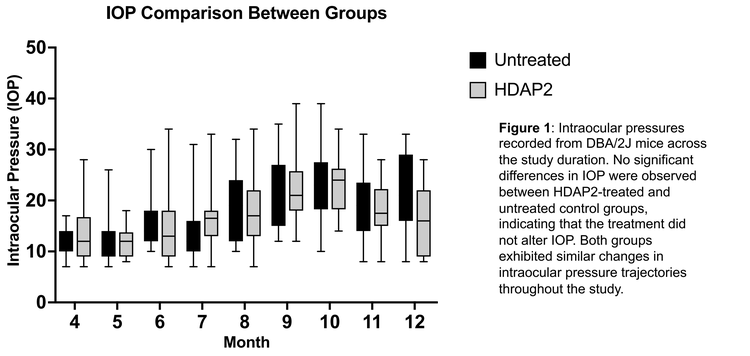

Figure 1: Intraocular pressures recorded from DBA/2J mice across the study duration. No significant differences in IOP were observed between HDAP2-treated and untreated control groups, indicating that the treatment did not alter IOP. Both groups exhibited similar changes in intraocular pressure trajectories throughout the study.

RESULTS

2. HDAP2 treatment significantly increased RGC survival.

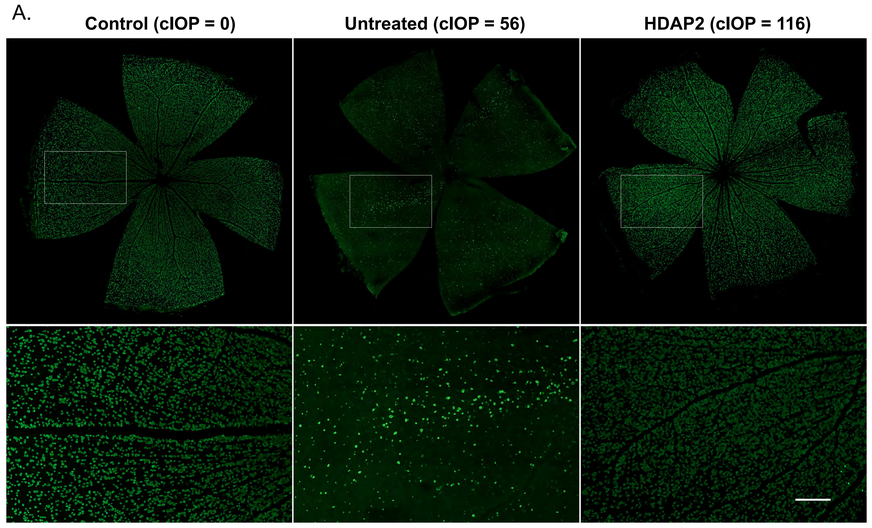

Figure 2A: Whole-mounted retinas stained for RGCs using rabbit anti-RBPMS (green). Insets show high magnification labeling. Untreated animals with cIOPs > 45 mmHg lost about 90% of RGCs (middle), while HDAP2-treated retinas (right) had RGC counts similar to controls. Scale = 100 µm.

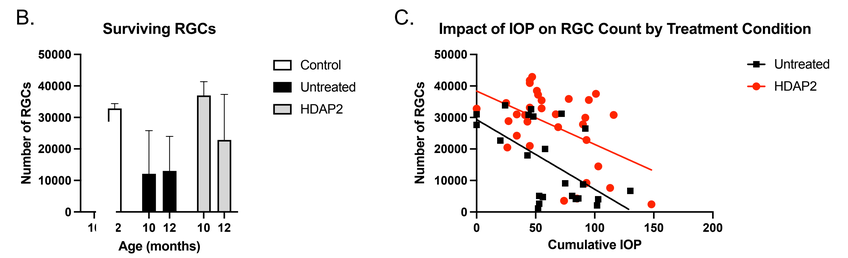

Figure 2B. Mean RGC counts in Control, Untreated, and HDAP2-treated animals at 10 and 12 months. At 10 months, one-way ANOVA revealed a significant effect of condition ($F(2, 16) = 26.55$, $p = 1.72 \times 10^{-5}$), and Tukey tests confirmed significantly higher RGC counts in Control vs. Untreated and Control vs. HDAP2 ($p < 0.001$), with a trend toward increased survival in HDAP2 vs. Untreated ($p = 0.063$). A Welch's t-test confirmed significantly greater RGC survival in HDAP2-treated animals compared to Untreated ($t = 4.27$, $p = 0.0055$). No significant differences were observed at 12 months. **Figure 2C.** Linear regression of RGC counts as a function of cumulative high IOP (cIOP), which was calculated by summing only the IOP values that were ≥ 20 mmHg at each time point. Each point represents one retina. Solid lines show best-fit linear regressions for each group. Slopes did not differ significantly ($p = 0.64$), but overall RGC counts were significantly higher in the HDAP2 group ($p < 0.001$).

3. Mitochondria in HDAP2-treated animals are more frequently encountered and contain more cristae than in Untreated animals.

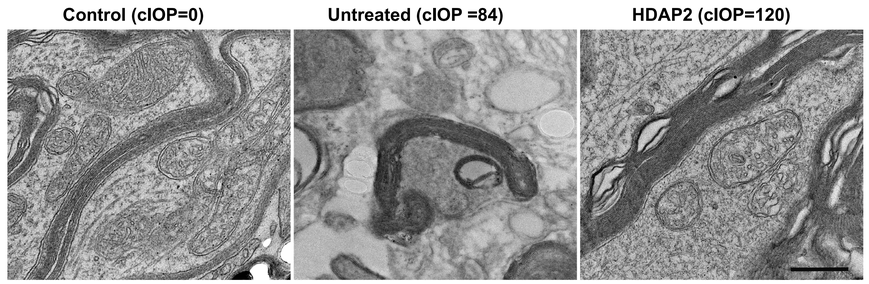

Figure 3: Mitochondria in optic nerve axons from Control (left), Untreated (center), and HDAP2-treated (right) animals. Mitochondria in HDAP2-treated nerves appeared similar in shape and density to those in control nerves and contained many cristae. Untreated axons had few mitochondria, disrupted myelin sheaths and opaque, disorganized axoplasm. Scale = 500 nm.

RESULTS

4. HDAP2 protects optic nerve morphology, axon number and area.

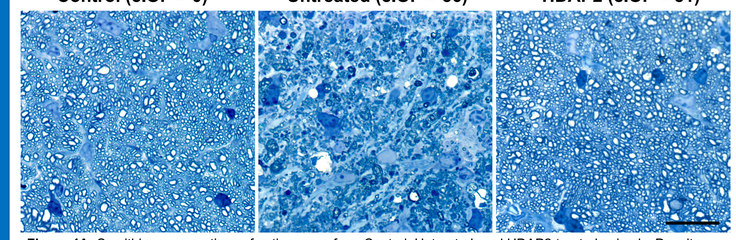

Figure 4A: Semithin cross-sections of optic nerves from Control, Untreated, and HDAP2-treated animals. Despite similarly elevated cIOPs in the Untreated and HDAP2-treated groups, only the HDAP2-treated nerves exhibited preserved axon morphology. Scale = 20 µm.

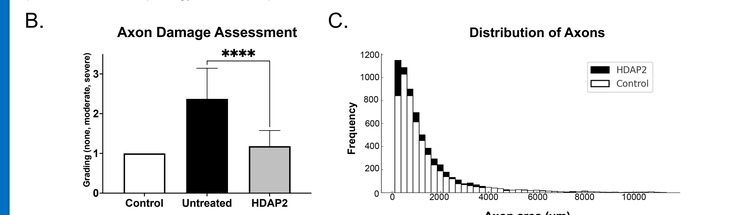

Figure 4B. Optic nerves from Control, Untreated, and HDAP2-treated animals with cumulative IOPs >45 mmHg were scored for axon degeneration severity on a scale of 1 (no degeneration), 2 (moderately degenerated), or 3 (degenerated). A Kruskal-Wallis H-test revealed a highly significant difference in axon grading among the groups ($H = 37.41$, $p = 7.5 \times 10^{-9}$). **Figure 4C.** Axon counts and cross-sectional areas were quantified in Control and HDAP2-treated nerves using the AxonJ plugin for ImageJ. In HDAP2-treated animals, both axon number and area were comparable to those in a 3-month-old control nerve. In contrast, the nerve from a 12-month-old untreated animal contained too few axons to quantify reliably.

CONCLUSIONS

Long-term HDAP2 treatment in DBA/2J mice with elevated intraocular pressure:

1. Significantly increased the likelihood of retinal ganglion cell survival.
2. Preserved optic nerve integrity, suggesting maintained axonal transport in HDAP2-treated animals.
3. Preserved mitochondrial morphology compared to untreated controls, indicating sustained ATP production capacity under elevated IOP.
4. Exhibited age-dependent efficacy, with younger animals demonstrating greater resistance to pressure-induced damage than older animals.

NEXT STEPS

Following the results presented here, we plan to:

1. Perform a detailed quantitative analysis of mitochondrial morphology, including assessments of cristae density, structural integrity, and fragmentation.
2. Develop predictive models to investigate the relationship between intraocular pressure and retinal ganglion cell survival.
3. Evaluate visual function in HDAP2-treated animals by recording visually evoked potentials.

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