**Neuroprotective properties of α-B-Crystallin core peptide (ABCP)**

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**Introduction**

Elevated intracocular pressure (IOP) is the major risk factor for vision loss due to degeneration of retinal ganglion cells (RGCs) in glaucoma. RGCs receive visual information from photoreceptors and that information is then sent to the brain. When RGCs deteriorate, vision weakens and leads to permanent loss of vision. Our recent studies show that αB-crystallin core peptide (ABCP) can be used to prevent this degeneration in RGCs thereby impeding the neurodegenerative changes occurring in glaucoma. In this experiment, we tested the effects of ABCP in RGCs and the scramble peptide was used as non-specific control. The main goal of this experiment was to test the neuroprotective effects of the ABCP peptide on isolated primary RGCs as well as whole retinal explants in hypoxic condition.

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**Results**

**α-B-Crystallin core peptide (ABCP) protects primary postnatal d7 rat RGCs from hypoxia induced cell death**

![Image 1](image1)

**Figure 1**: Images show the results of ABCP treated with scrambled peptide and ABCP peptide when cultured in a normoxic condition. ABCP treated cells presented no dead cells. However, the scrambled peptide groups showed some dead RGCs.

**Percentage of Dead Cells in Normoxia & Hypoxia**

![Percentage of Dead Cells in Normoxia & Hypoxia](image2)

**Figure 3**: ABCP peptide showed significant protection of primary RGCs from hypoxic insult.  *p<0.05, All Pairwise Multiple Comparison Procedures (Dunn’s Method).**

**α-B-Crystallin core peptide (ABCP) protects adult rat RGCs from hypoxia induced cell death**

![Image 4](image4)

**Figure 4**: Scrambled peptide and vehicle treated explants were placed in a normoxic environment to determine if the vehicle is not causing detrimental effects to the RGCs. Images show that the RGCs (Brn3a labeled, indicated in green) in the explants were unaffected by scramble peptide control in normoxic conditions.

**Hypoxia**

![Hypoxia](image5)

**Figure 5**: The explants were cultured in low oxygen levels for 16 hours. Retinal explants were labelled with retinal ganglion cell marker Brn3a in green, to determine changes in their viability. ABCP peptide at 13.5 µg/mL, was able to protect RGCs against hypoxic insult suggestive of its neuroprotective capabilities.

**RGCs / mm² in adult retinal explants**

![RGCs / mm² in adult retinal explants](image6)

**Figure 6**: Quantification of RGC counts per mm². ABCP is able to significantly protect adult retinal ganglion cells from dying by 3.5 fold. Vehicle and Scrambled peptide showed no significant difference in RGC number in a hypoxic condition and resulted in RGC death.  * indicates p<0.05, All Pairwise Multiple Comparison Procedures (Dunn’s Method).**

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**Methods**

**RGCs isolation**: Retinas were extracted from 7 day old Sprague Dawley rat pups and left in 4.5 units/ml of papain solution for 30 minutes at 37°C. Primary RGCs were then isolated from the retina by disassociation. Primary RGCs were then isolated from the retina by disassociation. Primary RGCs were then isolated from the retina by disassociation.

**Treatments**: After maturation, these cells were exposed to two different environments at 10% CO2 and 0.5% O2. In the normoxic condition, the explants were incubated with the cells for 30 minutes at 37°C. Another condition exposed the cells to a hypoxic environment (low levels of O2) at 10% CO2 and 0.5% O2 at 37°C. Cells were treated with either ABCP (12.5 µg/mL) or scramble peptide (12.5 µg/mL).

**Immunostaining**: Explants were fixed with 4% paraformaldehyde overnight at 4°C and then permeabilized with buffer containing Triton X-100 for 10 min. Blocking buffer (1x PBS/5% normal goat serum/5% BSA) was applied to the explants and is left for 24 hours at 4°C. After blocking, the explants were incubated with antibody against Brn3a (1:500 dilution) for 48-72 hours at 4°C. Explants were then washed three times with PBS and mounted on unifrost microscopic slides and left in the dark overnight until imaging.

**Imaging**: The retinal explants were observed using Zeiss LSM 510 Meta scanning confocal microscope at 40x magnification. Images were taken and cells were counted and data subjected to statistical analysis. The scale bar is adjusted to 50 µm.

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**Conclusions**

- Hypoxic environment was toxic to RGCs and caused cell death.
- ABCP at 12.5 µg/mL had significant neuroprotective effects to postnatal d7 and adult RGCs when exposed to a hypoxic environment.

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**REFERENCES**


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