Interactions of Elovanoids with G-Protein Coupled Receptor 120 in Retinal Pigment Epithelial Cells

Hamza Aallouche
Gethein Andrew Nicolas G. Bazan
Neuroscience Center of Excellence, School of Medicine
LSU Health New Orleans, New Orleans LA

Abstract

Dry age-related macular degeneration (AMD) is caused by the chronic degeneration of the macula due to drusen deposits in the retina and is the most common cause of vision loss in people over the age of 50. The cause of dry AMD is still unknown but may be caused by the depletion of protective lipids in the retina, which increases oxidative stress in the retina. Treatments for dry AMD are limited but recently discovered neuroprotective lipid mediators, Elovanoids (ELVs), may be useful in combating AMD. ELVs are very long-chain polyunsaturated fatty acids (VLC-PUFAS) that have been hydroxylated to have two alcohol groups. ELVs have been found to protect Retinal Pigment Epithelial (RPE) cells in the retina but the mechanism of this protective effect is unknown.

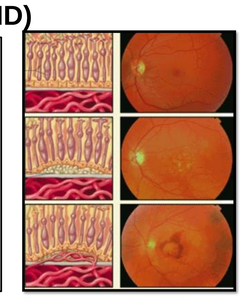
We are investigating the mechanism of Elovanoid's protective effect on RPE cells using immunocytochemistry (ICC) and Incucyte live cell imaging. Our experiment investigates whether ELVs interact with G-Protein Coupled Receptor 120 (GPR120) using different visualization methods in RPE cells. 8-well chambers were prepared at a density of 15K cells/well. 2 wells were supplemented with ELV-32, 2 were given GPR120 antagonist (AH 7614), and 2 were given GPR120 agonist (GSK137647). ICC was performed to visualize GPR120 surface expression after varying levels of stimulation with each ligand.

Incucyte live cell imaging was used to test the impact of ELVs on RPE cells and the lipid's ability to protect or prevent damage under uncompensated oxidative stress (UOS). 96 well plates of cells were given varying concentrations of hydrogen peroxide while also given ELVs, known antioxidants, Omega-3 fatty acids, and controls to investigate the effect of ELVs on cells with varying levels of uncompensated oxidative stress. The same treatment was used on GPR120 silenced, non-transfected, and negative control RPE cell lines to investigate and compare results to elucidate the role of ELVs on RPE cells in inactivated GPR120. The purpose of these experiments is to determine if ELVs influence the activation or inhibition of GPR120 to aid their protective abilities in RPE cells and determine if GPR120 is pro- or anti-apoptotic. We are investigating GPR120's role in RPE cell protection and if the receptor binds ELVs to better protect against UOS.

Introduction

Age Related Macular Degeneration (AMD)

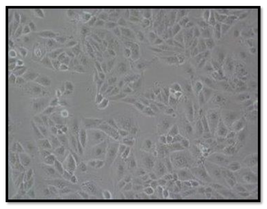
•Dry age-related macular degeneration (AMD) is the most common cause of irreversible vision loss in people over the age of 50.
•Patients with dry AMD experience dysfunction in the RPE/photoreceptor interaction.
•There are currently no treatments for dry AMD.[1]
•Treatments for wet AMD include injecting antibodies into the vitreous of the eye to prevent Vascular Endothelial Growth factor (VEGF) from promoting further growth of blood vessels in the retina.[2]



Top to bottom Normal, Dry, Wet.
Courtesy of healthplexus.net

Retinal Pigment Epithelial Cells (RPE)

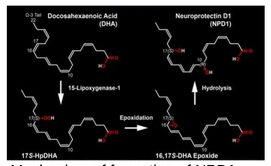
•RPE cells are pigmented cells found in the outermost layer of the retina.
• They support photoreceptor function and act as a barrier for the photoreceptors with their tight junctions formed with occludin and claudin.RPE cells also transport nutrients, electrolytes, and water from the choroid cappillaris and phagocytize the top segments of photoreceptors daily.[3]
•Excessive light induces photooxidation which breaks down molecules and generate free radicals which cause oxidative damage. As a result, RPE cells have antioxidant systems which aim to counteract this oxidative damage such as the Glutathione system, Superoxide Dismutase, etc.[4]



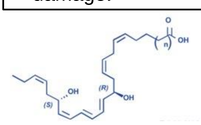
Brightfield image of RPE cells
Courtesy of Gethein

Elovanoids (ELVs)

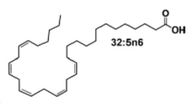
- ELVs are very long-chain polyunsaturated fatty acids (VLC-PUFAS) that have been hydroxylated to have two alcohol groups. They have been found to protect RPE cells from oxidative stress damage.[5,6]



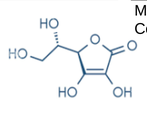
Mechanism of formation of NPD1.
Courtesy of the Bazan Lab



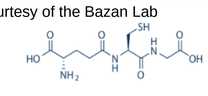
Elovanoid



VLC-PUFA-32



Vitamin C (ascorbic acid)



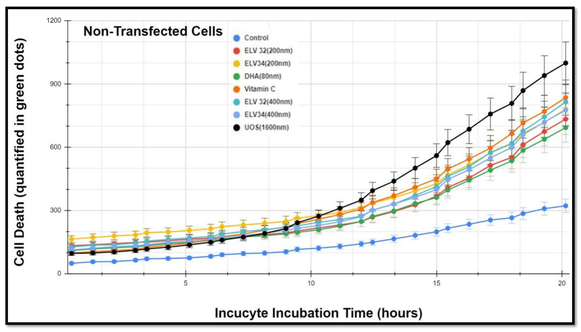
Glutathione

Hypothesis

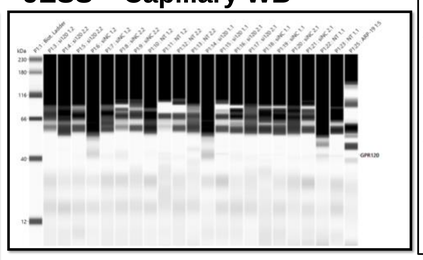
- **Overall Hypothesis: Elovanoids interact with the G-Protein Coupled Receptor-120 (GPCR-120)**
- **Aim 1) Elovanoids act as agonists to GPR120**
- **Aim 2) Elovanoids protect RPE cells from cell death during excessive oxidative stress.**

Results

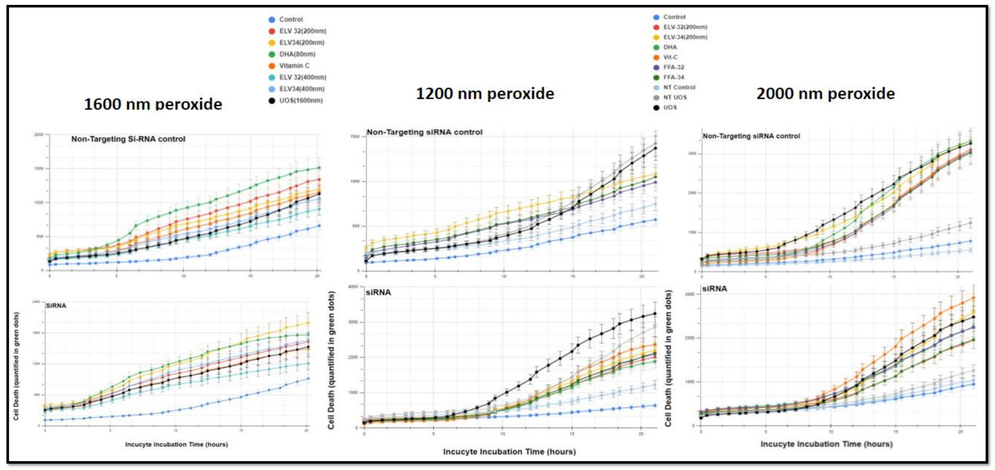
Incucyte Live Cell Imaging



JESS – Capillary WB

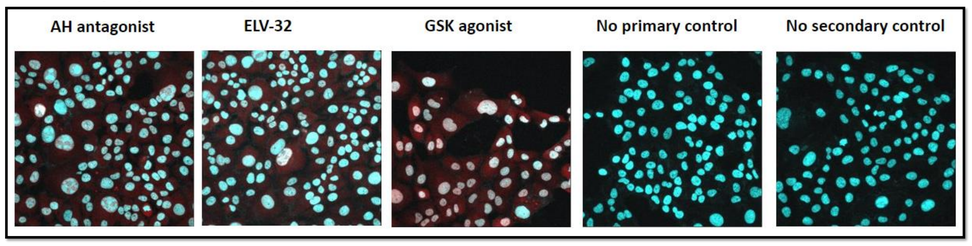


•Capillary western blot (WB)
•More sensitive than traditional WB
•Uses a small amount of protein
•Used to confirm protein is silenced

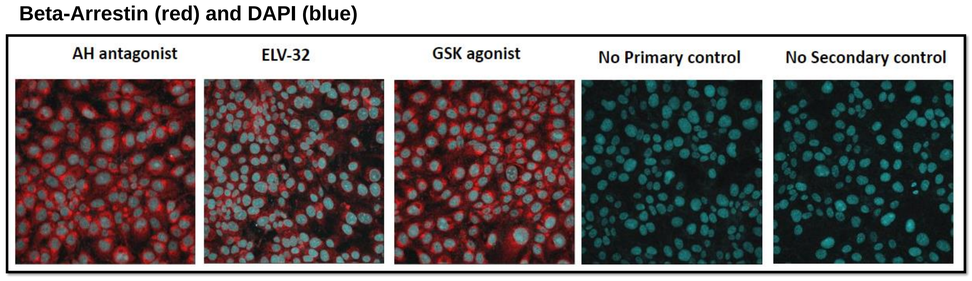


Immunocytochemistry

GPR120 (red) and DAPI (blue)



Beta-Arrestin (red) and DAPI (blue)

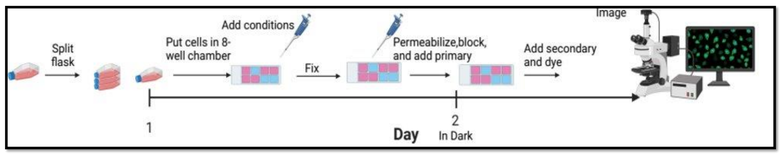


Conclusions

Incucyte:
•When GPR120 is expressed in RPE cells, ELVs at all concentrations prevent cell death
•DHA, a known GPR120 agonist, elicits the strongest cellular protection.
•When GPR120 is silenced, ELVs at EC50 and DHA exhibited cell death or similar to UOS.
•At extreme UOS concentrations, all conditions were futile in preventing cell death.
ICC:
•Elovanoids did not have a significant impact on GPR120 surface expression compared to known agonists.

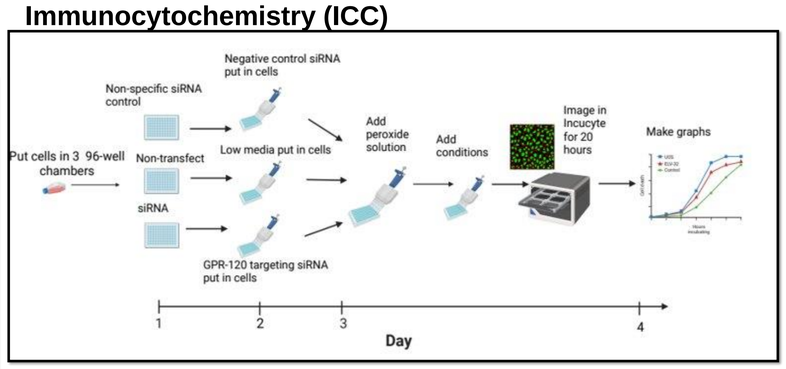
Methods

Incucyte – Live Cell Imaging

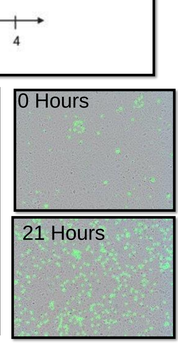


•8 well chambers at a cell density of 15K in 250 µL
lipids, agonist, antagonist, were added and left in cells for 10 minutes. They are then fixed with paraformaldehyde.
•Cells were then Permeabilized with Triton-X to poke holes in the cell membrane and Blocked with PBS and BSA.
• Primary antibody were added for staining for GPCR120 or Beta Arrestin and left at 4C for a day
•Then secondary antibody and the Hoechst 568 dye were added to cells and are left at 4C. This is done in the dark as the secondary antibody and the Hoechst stain can be damaged by light.
•The chambers are then left in 4C until ready to image with ICC

Immunocytochemistry (ICC)



- 96 well chambers for 3 different H2O2 concentrations were prepared at 20K cell concentration with non-transfect, siRNA, and GPR120 expressed cells.
- 2 controls, 2 UOS, 200nm 32, 200nm 34, 400nm 32, 400 nm 34, 80 nm DHA, Vitamin C, and known agonists and antagonists of GPR 120 were given for 1600 peroxide concentration.
- For 1200 and 2000 concentration H2O2 ELV 32 200, ELV34 200, 200 nm DHA, Vit C, FFA-32,FFA-34, 2 UOS, and 2 control, and control and UOS non transfect.



Green dots show the cell death in a well over time with Styox-Green

References

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