Sigma-2 receptor modulators rescue POS trafficking deficits in RPE cell-based models of dry AMD

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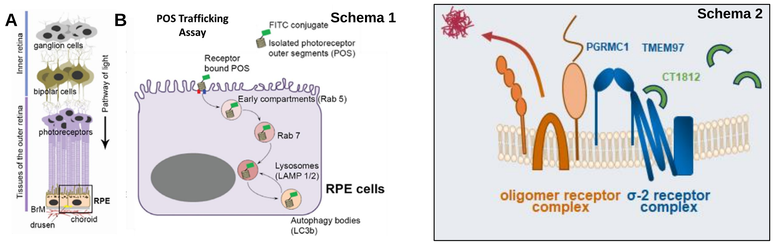
INTRODUCTION

Age-related macular degeneration (AMD) is one of the leading causes of blindness. While treatments exist for wet AMD, there are no approved therapeutics for dry AMD (dAMD), which comprises 90% of all AMD cases[1]. There are several hallmarks of dAMD including inflammation, oxidative stress, and the presence of amyloid-beta oligomers (AβO), which can disrupt key homeostatic functions of retinal pigmented epithelium (RPE) cells, including the ability of RPE cells to phagocytose or recycle photoreceptor outer segments (POS; **Schema 1**)[2-5]. The sigma-2 receptor (S2R), encoded by TMEM97, is a damage sensor, regulating processes disrupted in age-related diseases[6]. Genome-wide association studies (GWAS) indicate a single nucleotide polymorphism (SNP) exists in the TMEM97 locus that confers a decreased risk of dAMD[7-8], and down-regulation of TMEM97 expression protects RPE cells from oxidative stress[9]. The S2R modulator CT1812 is currently in Phase 2 clinical trials for Alzheimer's disease and dementia with Lewy bodies, although CT1812 is not yet approved for any indication. Proteomic analyses from aged patients given CT1812 or placebo suggest S2R modulators regulate key proteins and pathways disrupted in dAMD[10-11]. S2R modulators prevent AβO from binding to neurons (**Schema 2**) and rescue deficits in neuronal functioning[6], but whether S2R modulators can limit injury from other stressors has not been investigated. Based on the role of the S2R as a key damage sensor and the link of S2R to dAMD, the hypothesis that S2R modulators could rescue AβO and oxidative stress-induced deficits was tested by assessing the ability of RPE cells to phagocytose / recycle POS (**Schema 1**).



Schema 1. A) Pathway of light from ganglion cells to RPE with drusen. **B)** POS pulsed feeding assay. FITC conjugated POS are trafficked through RPE cells via lysosomes (labeled with LAMP2) and autophagy bodies (labeled with LC3B)[2].Co-localization of POS to lysosomes or autophagy bodies can be measured to assess normal trafficking of POS and disruptions caused by disease-relevant stressors.

Schema 2. The S2R (blue) comprises TMEM97 and PGRMC1. A representation of the S2R modulator CT1812 (green) displacing AβO (red) from binding neuronal synapses is shown[6].

AβO bind to RPE cells in a concentration and time-dependent manner

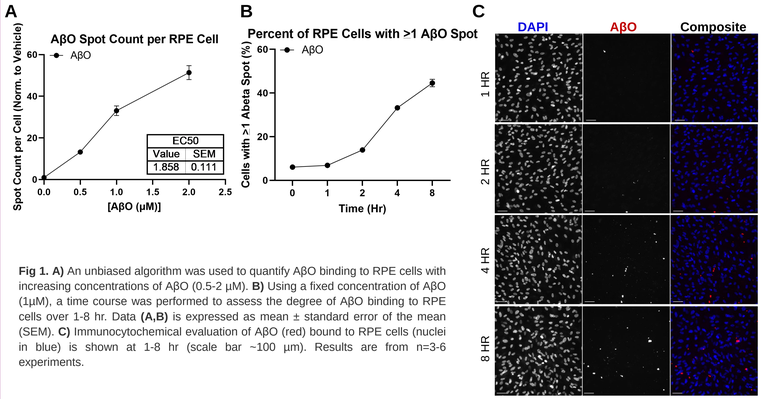


Fig 1. A) An unbiased algorithm was used to quantify AβO binding to RPE cells with increasing concentrations of AβO (0.5-2 μM). **B)** Using a fixed concentration of AβO (1μM), a time course was performed to assess the degree of AβO binding to RPE cells over 1-8 hr. Data **(A,B)** is expressed as mean ± standard error of the mean (SEM). **C)** Immunocytochemical evaluation of AβO (red) bound to RPE cells (nuclei in blue) is shown at 1-8 hr (scale bar ~100 μm). Results are from n=3-6 experiments.

A third chemically distinct S2R modulator rescues AβO and H_2O_2-induced deficits in POS trafficking

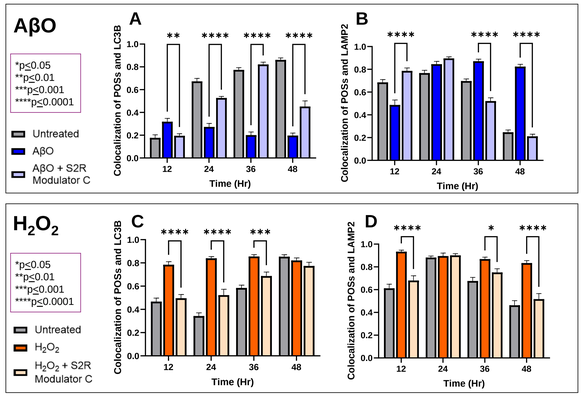
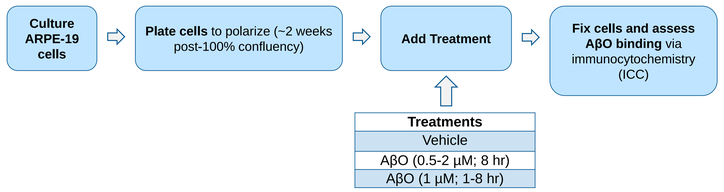


Fig 4. An unbiased algorithm was used to quantify **(A)** LC3B or **(B)** LAMP2 and POS colocalization after 1 μM AβO exposure (12-48 hr) in the presence and absence of the S2R modulator C. A significant rescue in the AβO-induced deficit was observed for LC3B with S2R modulator C at 12, 24, 36, and 48 hr (p<0.01), and for LAMP2 at 12, 36, and 48 hr (p<0.0001). Similarly, the colocalization of **(C)** LC3B or **(D)** LAMP2 with POS was assessed after exposure to 100 μM H_2O_2 (12-48 hr) in presence and absence of the S2R modulator. A significant rescue in the H_2O_2-induced deficit with S2R modulator C was observed for LC3B at 12, 24, and 36 hr (p<0.001), and for LAMP2 at 12, 36, and 48 hr (p<0.05). Statistical significance was assessed via 2-way ANOVA and Tukey's post-hoc test (n=3 from 1 experiment). Data is expressed as mean ± standard error of the mean (SEM).

METHODS

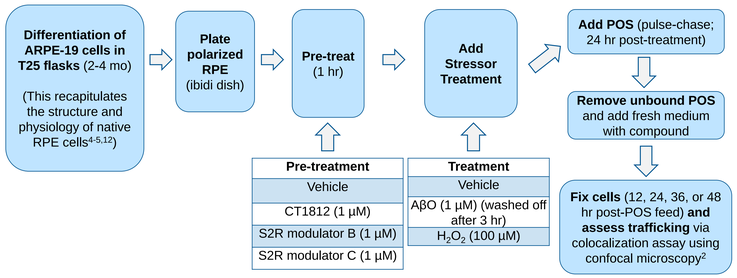
Goal 1) To characterize AβO binding in RPE cells

Experimental Design: Concentration-response and time course of AβO binding in RPE cells



Goal 2) To determine whether S2R modulators rescue AβO and H_2O_2-induced deficits in the ability of RPE cells to phagocytose POS

Experimental Design: Examination of POS trafficking over time in polarized RPE cells



S2R modulators, including CT1812, rescue AβO-induced deficits in the homeostatic recycling of POS

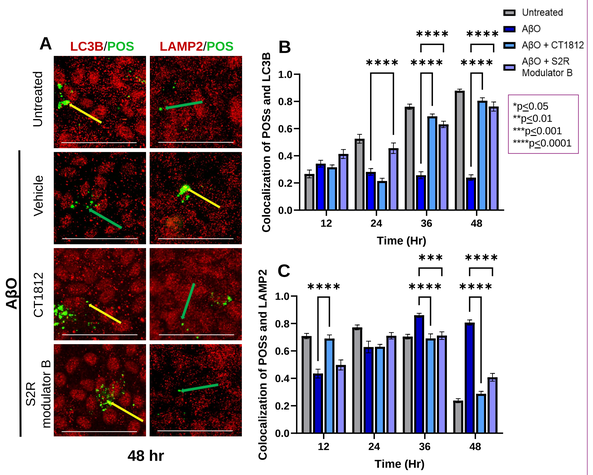


Fig 2. A) Confocal microscopy was used to measure colocalization (yellow) of LC3B (red) and LAMP2 (red) with POS (green) at 48 hr (scale bars ~50 μm). Arrows point to colocalization (yellow) or lack thereof (green). An unbiased algorithm was used to quantify **(B)** LC3B or **(C)** LAMP2 and POS colocalization after 1 μM AβO exposure (12-48 hr) in the presence and absence of a S2R modulator. **B)** A significant increase in LC3B-POS colocalization towards control levels was observed with CT1812 at 36 and 48 hr (p<0.0001), and with S2R modulator B at 24, 36, and 48 hr (p<0.0001). **C)** A significant reduction in LAMP2-POS colocalization towards control levels was observed with CT1812 at 12, 36, and 48 hr (p<0.0001), and with S2R modulator B at 36 and 48 hr (p<0.001). Statistical significance was assessed via 2-way ANOVA and Tukey's post-hoc test (n=5 wells from 2 experiments). Data are expressed as mean ± standard error of the mean (SEM).

S2R modulators, including CT1812, rescue H_2O_2-induced deficits in the homeostatic recycling of POS

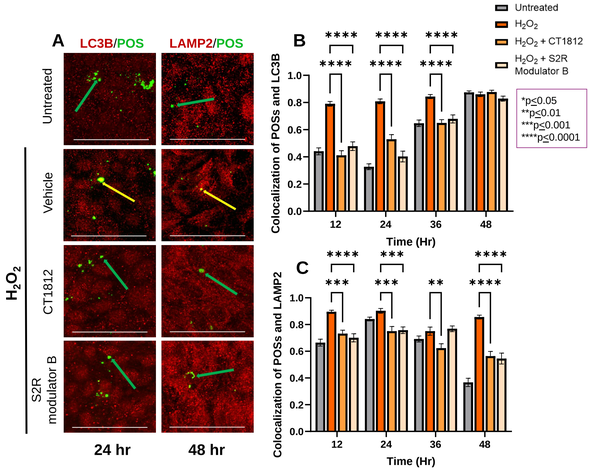
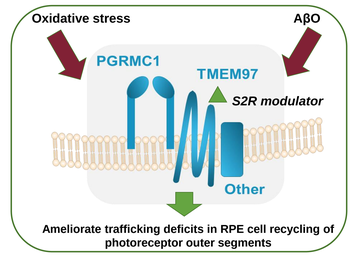


Fig 3. A) Confocal microscopy was used to measure colocalization (yellow) of LC3B (red) and LAMP2 (red) with POS (green) at 24 hr and 48 hr, respectively (scale bars ~50 μm). Arrows point to colocalization (yellow) or lack thereof (green). An unbiased algorithm was used to quantify **(B)** LC3B or **(C)** LAMP2 and POS colocalization after 100 μM H_2O_2 exposure (12-48 hr) in the presence and absence of a S2R modulator. **B)** A significant reduction in LC3B-POS colocalization towards control levels was observed with CT1812 at 12, 24, and 36 hr (p<0.0001), and with S2R modulator B at 12, 24, and 36 hr (p<0.0001). **C)** A significant reduction in LAMP2-POS colocalization towards control levels was observed with CT1812 at 12, 24, 36, and 48 hr (p<0.01), and with S2R modulator B at 12, 24, and 48 (p<0.001). Statistical significance was assessed via 2-way ANOVA and Tukey's post-hoc test (n=5 wells from 2 experiments). Data are expressed as mean ± standard error of the mean (SEM).

CONCLUSIONS

- Oxidative stress and toxic AβO disrupt trafficking of photoreceptor outer segments (POS) in RPE cells
- S2R modulators, from three chemically distinct series, rescue oxidative stress and AβO-induced deficits in POS trafficking, normalizing a key homeostatic process disrupted in dAMD
- Results expand beyond the previously elaborated mechanism of action of CT1812 and suggest CT1812 may be protective against oxidative stress, as well as AβO toxicity, in age-related degenerative diseases



Genetic evidence, clinical biomarker, and preclinical data suggest that modulation of S2R may be a promising approach to treat dAMD and supports advancing CT1812 to a Ph2 clinical trial in dAMD

REFERENCES
1. Rabin, D. M. et al. (2013). Chronic oxidative stress upregulates Drusen-related protein expression in adult human RPE stem cell-derived RPE cells: a novel culture model for dry AMD. *Aging*, *5*(1), 51–66.
2. Keeling, E. et al. (2019). Oxidative Stress and Dysfunctional Intracellular Traffic Linked to an Unhealthy Diet Results in Impaired Cargo Transport in the Retinal Pigment Epithelium (RPE). *Molecular nutrition & food research*, *63*(15), e1800951.
3. Liu, R. T. et al. (2013). Inflammatory mediators induced by amyloid-beta in the retina and RPE in vivo: implications for inflammasome activation in age-related macular degeneration. *Investigative ophthalmology & visual science*, *54*(3), 2225–2237.
4. Lynn, S. A. et al. (2021). Oligomeric $A\beta_{1-42}$ Induces an AMD-Like Phenotype and Accumulates in Lysosomes to Impair RPE Function. *Cells*, *10*(2), 413.
5. Lynn, S. A. et al. (2017). Ex-vivo models of the Retinal Pigment Epithelium (RPE) in long-term culture faithfully recapitulate key structural and physiological features of native RPE. *Tissue & cell*, *49*(4), 447–460.
6. Izzo, N. J. et al. (2014). Alzheimer's therapeutics targeting amyloid beta 1-42 oligomers II: Sigma-2/PGRMC1 receptors mediate Abeta 42 oligomer binding and synaptotoxicity. *PloS one*, *9*(11), e111899.
7. Fritsche L.G, et al. (2016). *A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants.* Nat Genet. 48(2):134-43.
8. Logue, M. W. et al. (2013).*A search for age-related macular degeneration risk variants in Alzheimer disease genes and pathways.* Neurobiology of Aging. Retrieved April 11, 2022.
9. Wang, J.-H. et al. (2020). *Functional study of the AMD-associated gene TMEM97 in retinal pigmented epithelium using CRISPR interference.* BioRxiv. Retrieved April 11, 2022.
10. Hamby, M.E. (2022, March 15-20). *Proteomic analyses of CSF in a Phase 2 clinical trial for AD to identify pharmacodynamic biomarkers of the S2R modulator CT1812.* [Poster]. ADPD, Barcelona, Spain.
11. Waybright, L. (2021, May 1-7). *Proteomic analyses of CSF in a Phase 2 clinical trial for AD to identify pharmacodynamic biomarkers of the S2R modulator CT1812.* [Poster]. ARVO, virtual
12. Lynn, S. A. et al. (2018). A convenient protocol for establishing a human cell culture model of the outer retina. *F1000Research*, *7*, 1107.

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