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

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Abstract

Purpose : Toxic amyloid-beta oligomers (AβOs) and oxidative stress are hallmarks of dry age-related macular degeneration (dAMD) and can disrupt key homeostatic processes in retinal pigmented epithelium (RPE) cells (Rabin et al. 2013). AβOs disrupt RPE cell function *in vivo* (Ruozhou et al. 2013) and normal RPE-mediated photoreceptor outer segment (POS) phagocytosis *in vitro* (Lynn et al. 2021). Sigma-2 receptor (S2R) modulators prevent AβOs from binding to neurons and rescue deficits in neuronal functioning (Izzo et al. 2014). Based on dAMD-related deficits in RPE function and the role of the S2R as a key damage sensor, the hypothesis that S2R modulators could rescue AβO and oxidative stress-induced deficits in the ability of RPE cells to phagocytose POSs was tested.

Methods : A human RPE cell line, ARPE-19 cells, were exposed to AβOs (0.5-2μM) over time (1-8 hr) and AβO binding was assessed via immunocytochemistry (ICC) and high content imaging (n=3-6 experiments). A trafficking assay was used to monitor internalization and degradation of POS over time. ARPE-19s were exposed to AβOs or H_2O_2 in the presence or absence of S2R modulators. The effects of S2R modulators on POS colocalization with lysosomal associated membrane protein 2 (LAMP2) and microtubule-associated proteins 1A/1B light chain 3B (LC3B) were quantified via confocal imaging and unbiased algorithm across time (12-48 hr; n=2 experiments). Statistical significance ($p<0.05$) was determined via Two-Way ANOVA and *post hoc* Tukey's test.

Results : ICC studies show that AβOs bind to ARPE-19s in time and concentration-dependent manners (EC50=1.86μM). Following exposure of cells to 1μM AβO, POS are retained in LAMP2 positive vesicles and trafficking is diminished to LC3B vesicles when compared to control. S2R modulators from three independent chemical series restored POS trafficking through LAMP2 positive vesicles at 36 and 48 hr ($p<0.0001$) and through LC3B vesicles at 48 hr ($p<0.0001$). The same S2R modulators restored POS trafficking after exposure to 100μM H_2O_2 through LAMP2 positive vesicles at 12 and 48 hr ($p<0.001$) and through LC3B vesicles at 12, 24, and 36 hr ($p<0.001$).

Conclusions : Results point to a role of S2R modulators in rescuing AβO and oxidative stress-induced deficits in RPE cells by normalizing the homeostatic recycling of POSs in RPE cells. These data support S2R modulators as a promising potential therapy for dAMD.

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