Prophylactic Treatment of Optimized Retinylamine Loaded PLA Particles in a Macular Degeneration Mouse Model

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Statement of Purpose: Ocular therapy encompasses a range of diseases including diseases of the retina, such as Stargardt disease and age-related macular degeneration (AMD). Current pharmacologic therapies available to AMD require intraocular injection or risk low ocular biodistribution due to the highly impervious barriers of the eye. The potentially dangerous side effects of this treatment warrant the need for new agents that can achieve therapeutic concentrations noninvasively prior to the progression of the disease. Reactive aldehydes, particularly the vitamin A aldehyde all-trans-retinal, are produced intrinsically from the visual chromophore 11-cis-retinal via metabolism within the retinoid cycle and require immediate clearance to prevent photoreceptor toxicity. Due to defective ATP binding cassette transporter 4 (ABCA4), inefficient clearance, as seen in aged mammalian visual systems, is associated with the accumulation of all-trans-retinal within the intradiscal region of the photoreceptor and poses the risk of additional toxic condensation products, such as A2E, to be formed[1]. ABCA4 mutations are found in Stargardt disease and AMD. Proof-of-concept therapies have begun with the development of RPE65 antagonists and the administration of Retinylamine[2] has been extensively examined for its therapeutic efficacy in model mice with the phenotype of retinal degeneration. However, a method for controlled and sustained release remains elusive for the delivery of retinopathy therapeutics, and thus is essential to be addressed.

Methods: PLA particles were formulated using a single emulsion/solvent evaporation method at [20mg/mL] polymer concentration and a 6.9% drug loading capacity. Particles were then subcutaneously injected with the minimal effective dose, 10mg/kg, into dark adapted *Abca4*$^{-/-}$*Rdh8*$^{-/-}$ double knockout mice (n=6) at predetermined time points prior to photobleach. Photobleach was carried out by intense yellow light at 10,000 lux for 30 minutes. Mice were then returned to the dark room for one week. Mice were analyzed utilizing both Optical Coherence Tomography (OCT) and Electroretinography (ERG) to investigate the state of the retina, specifically the state of the photoreceptor cells. Positive control groups were not photobleached and negative controls groups were photobleached but not treated. Further controls included treatment of free drug (10mg/kg) for comparison. Normal phase HPLC analysis of 11-cis-retinal was carried out at λ=325nm at a 90:10 Hexane:Ethyl Acetate ratio and flow rate of 1.4mL/min to establish overall rhodopsin levels. Severely degenerated retinas will have significantly reduced quantities of rhodopsin and therefore reduced levels of 11-cis-retinal.

Results: ERG and OCT analysis revealed that sustained release of Retinylamine prolonged protective effects to the retina as long as 7 days. Control quantities of both electrical signal (a-wave) and outer nuclear layer (ONL) thickness were conserved, whereas treatment of free Retinylamine showed similar levels for only 1 day and pronounced retinal degeneration at 6 days. These results were corroborated via HPLC analysis of 11-cis-retinal within retinas.

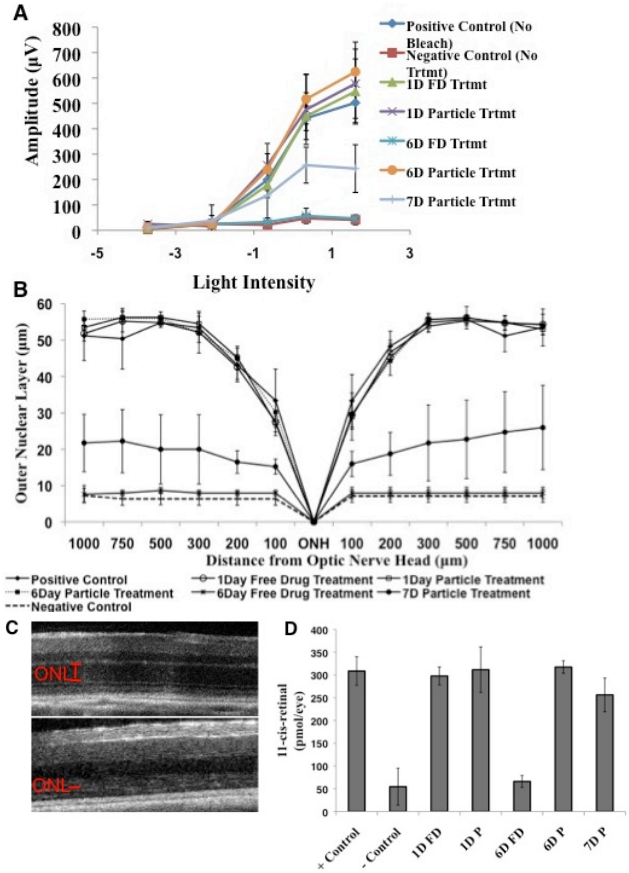

Figure 1: Retinal analysis via (a) ERG (a-wave) (b) OCT (ONL) (c) 6day OCT images, particle trtmt (top) free drug trtmt (bottom) (d) HPLC (11-cis-retinal).

Conclusions: The optimized PLA particle platform presented here significantly prolonged the protective effects of the ocular therapeutic, Retinylamine, at a minimal effective dosage. Controlled and sustained release platforms of therapeutics is optimal to any drug regimen and allows for complete patient compliance and maximal therapeutic efficacy throughout the duration of treatment while minimizing dosing occurrence. Future considerations could be taken to further prolong the release of this potent retinal therapeutic via polymeric conjugation methods.

References: [1]Maeda, A. JBC. 2009; 284:15173-15183.
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