INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of vision loss in people over the age of 50 in the developed world and is characterized by the formation of drusen. Drusen are deposits of cellular debris in the retinal pigment epithelium (RPE) and can induce the expression of the inflammasome which promotes the maturation of the inflammatory cytokines IL-1β and IL-18. Increased levels of IL-1β have been associated with products of oxidative stress such as 4-hydroxynonenal, microglia-RPE interactions, and lysosomal destabilization. Systemic injection of mice with NaIO3 has shown to be an effective way to induce oxidative stress in the RPE that results in retinal degeneration and pathology associated with geographic atrophy (dry AMD). Therefore, we utilized this acute model of oxidative stress to develop mice with AMD-like features. These mice were then used to test the efficacy of a soluble caspase activation and recruitment domain (CARD) in protecting mice from oxidative stress-induced retinal degeneration.

OBJECTIVES

1. Compare the electrophysiological function of the retina in eyes injected with AAV-GFP (control) to eyes injected with AAV-GFP-CARD (treatment).
2. Compare the thickness of various retinal layers in eyes injected with AAV-GFP to eyes injected with AAV-GFP-CARD.
3. Compare the morphology of the RPE of eyes injected with AAV-GFP to the RPE of eyes injected with AAV-GFP-CARD.
4. Observe whether systemic injection of NaIO3 is able to induce significant oxidative damage to the retina.

METHODS

1. Intravitreal injections: Rights eyes of five mice were injected with AAV-GFP and the left eyes were injected with AAV-GFP-CARD. 10⁶ vector genomes were injected per eye.
2. Induction of oxidative damage to the RPE: The same five mice were injected intraperitoneally with 30 mg/kg NaIO3.
3. Scopotic Electroretinography (ERG): a-wave, b-wave, and C-wave amplitudes were measured in the eyes of the mice at baseline (before AAV and NaIO3 injections) and at 1 week and 4 weeks post-injection.
4. Spectral Domain Optical Coherence Tomography (SD-OCT): Used to quantify the thickness of various retinal layers at 4 weeks post-injection in both control and treatment eyes.
5. RPE Flatmounts Immunostained with ZO-1: Used to observe the gross morphological structure of the RPE in both control and treatment injected eyes.

RESULTS

Figure 2: Electroretinography. At one week post-injection, there was significant degeneration in both eyes and no significant recovery in the GFP-CARD injected eye. However, by four weeks post-injection there was significant recovery in the CARD injected eyes compared to the GFP injected eyes. * = P < 0.05

Figure 3: Optical Coherence Tomography. There was a slight increased thickness in the total retina in GFP injected eyes compared to the GFP-CARD injected eyes four weeks post-injection. However, for most individual layers of the retina there was no significant difference in thickness between the GFP injected eyes and the CARD injected eyes.

DISCUSSION

1. In eyes where IL-1β activity was inhibited, electrophysiological function of the retina recovered significantly after four weeks following oxidative damage to the RPE.
2. Inhibition of IL-1β resulted in no appreciable difference in the thicknesses of various retinal layers as is often seen with AMD.
3. Inhibition of IL-1β injected eyes resulted in much less morphologically abnormal and destroyed RPE compared to eyes where IL-1β activity was not inhibited.
4. Delivery of a soluble CARD is capable of inhibiting the activation of IL-1β by mimicking the CARD domain of the NLRP3 inflammasome.
5. Inhibition of IL-1β activity shows some protection against oxidative damage to the RPE and the retinal degeneration that follows.

The AAV-delivered CARD used in this study will be used in a mouse model of dry AMD where the antioxidant enzyme superoxide dismutase 2 (SOD2) is deleted from the RPE. An AAV-based gene therapy against IL-1β may serve as a novel therapeutic agent for dry AMD.

REFERENCES