

Intravitreal triamcinolone acetate promotes rod and cone survival in RP

HENRY J KAPLAN MD, WEI WANG MD PHD, SAN JOON LEE MD, DOUGLAS C DEAN PHD

KY LIONS EYE CENTER, UNIVERSITY OF LOUISVILLE, KY

ST LOUIS UNIVERSITY SCHOOL OF MEDICINE, ST LOUIS, MO

KOSIN UNIVERSITY COLLEGE OF MEDICINE, BUSAN, KOREA

Financial Disclosure

No conflict exists in this presentation.

I have the following financial interests or relationships to disclose:

- Ally Therapeutics: Consultant/Advisor
- Caremark: Consultant/Advisor
- Regenexbio Inc: Consultant/Advisor
- Santen, Inc.: Consultant/Advisor
- Wave Life Sciences: Consultant/Advisor

Purpose/Summary

Cones are dependent upon rods for glucose transport and assembly of outer segments (OS). An unfolded protein response (UPR) initiated in response to ER stress, such as mutant rhodopsin (RHO), can lead to loss of rod photoreceptors and Retinitis Pigmentosa (RP). Retinal microglia phagocytosis initiates the demise of rods. We observed that the activation of microglia is inhibited by the intravitreal injection of a slow-release corticosteroid (triamcinolone acetate) [IVTA] and promotes rod and cone survival.

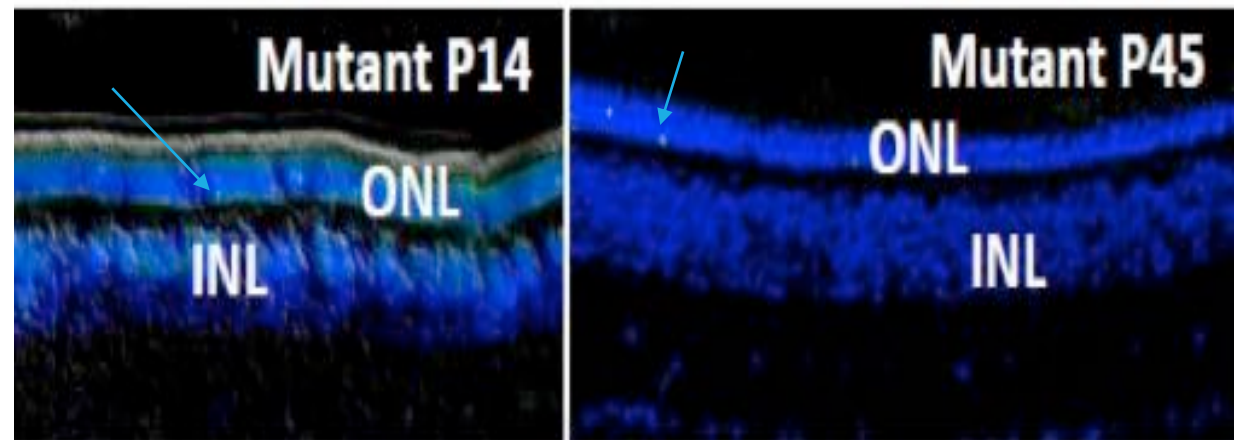
Methods

Wild type (WT) and P23H RHODOPSIN mutant RP pig littermates were followed for retinal apoptosis, expression of cytokines, microglial migration and engulfment of mutant rods at different ages and stages of retinal degeneration. The effects of IVTA injection at these ages and stages was compared. All of the changes (e.g., WT vs. RP and control vs. treated) in ERG, OKR, ONL rows and OS number exceeded statistical significance ≤ 0.05 .

Results - Rod loss in pig RP does not correlate with apoptosis

Because apoptosis can be triggered by chronic ER stress from misfolded proteins, we followed apoptosis during the time course of rod loss in the pigs. Although low levels of rod apoptosis (blue arrow) were evident regionally in the retina, this apoptosis did not correlate with or account for most rod loss during RP progression.

P23H RETINAL TUNNEL STAINING

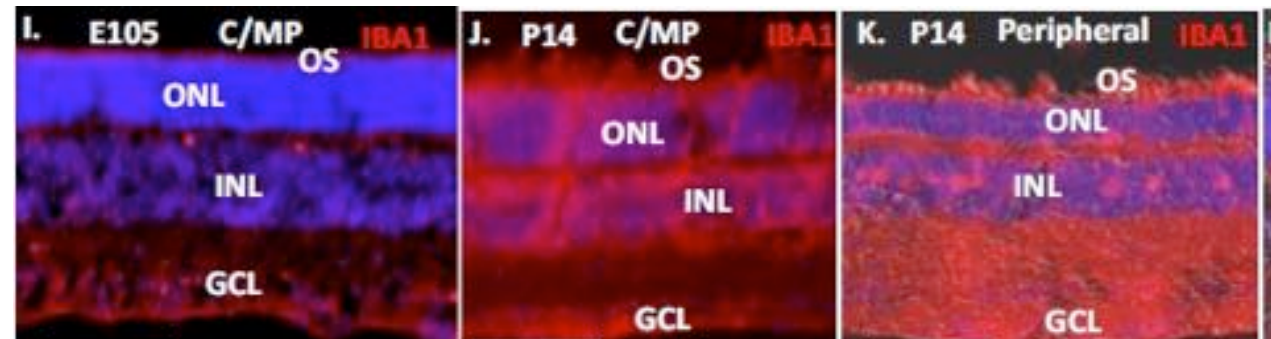


Programmed cell removal (PrCR) by microglia

The high percentage of mutant rods being phagocytosed by microglia appears to account for most rod loss during disease progression.

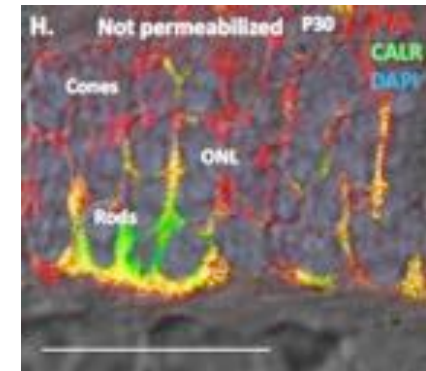
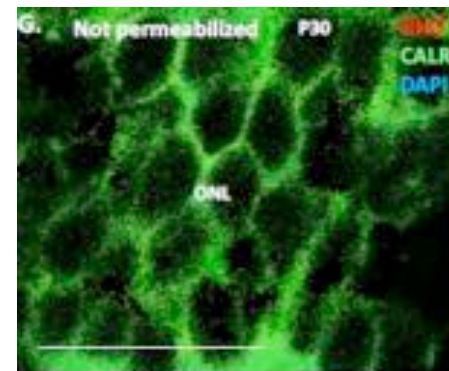
(I-K) Expression of IBA1 prior to birth at E105 and after birth at P14 in central, mid-peripheral and peripheral regions of RP pig retinas.

IMMUNOSTAINING SHOWING IBA1+ MICROGLIA



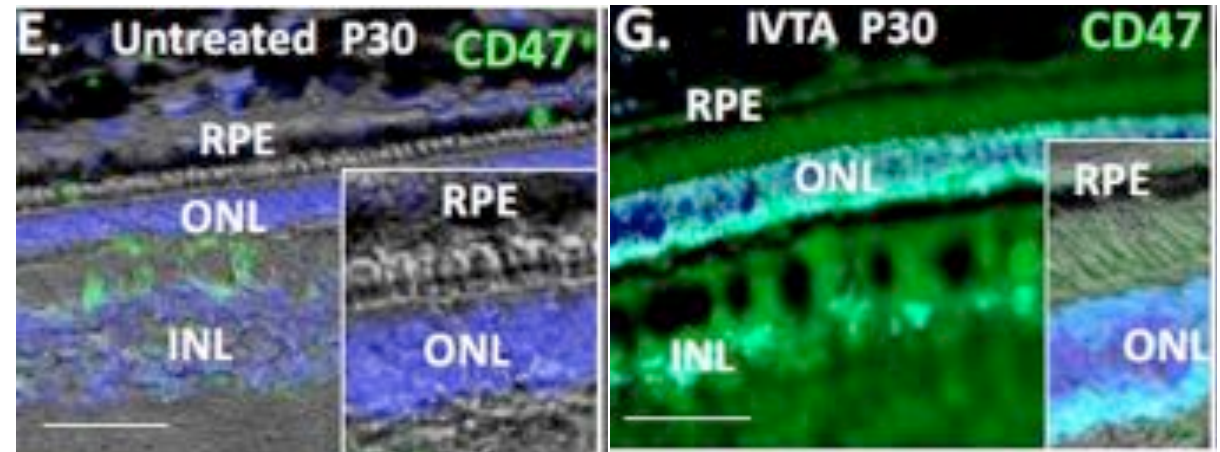
ER protein calreticulin (CALR) is a DAMP - i.e. an “eat me” recognition signal for microglia, induced by ER stress in RP

Upon ER stress, the ER protein calreticulin (CALR) translocates to the rod cell surface (G), where it serves as a damage-associated molecular pattern [DAMP], “eat me”, recognition signal for microglia. CALR co-localizes on the cell surface with CD73 (P73)(H).



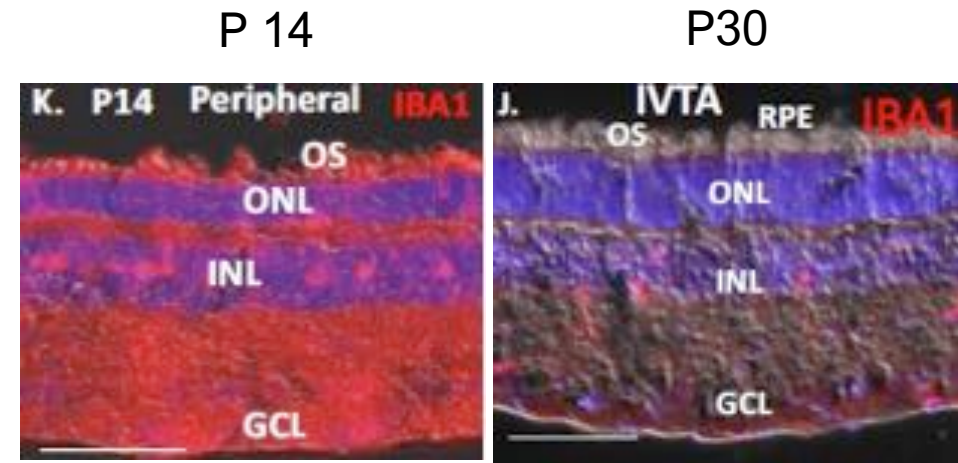
CD47 is an immune checkpoint - i.e. “don’t eat me” recognition signal for microglia, induced by IVTA

IVTA activates tyrosine receptor kinase B (TrkB) on rod surfaces establishing CD47 (P47) an immune checkpoint, “don’t eat me”, inhibition signal. CD47 is abundantly expressed (G) after IVTA but mostly absent (E) in untreated hosts.



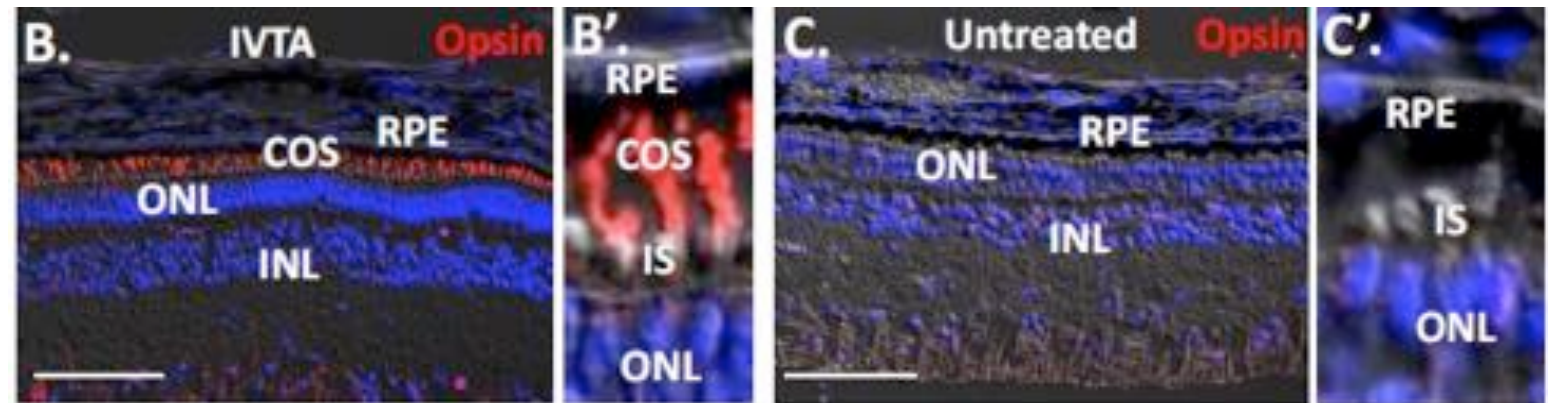
CD47 immune checkpoint, “don’t eat me”, inhibition signal prevents microglial activation

The CD47 immune checkpoint, “don’t eat me”, inhibition signal reduces expression of chemotactic/inflammatory cytokines and microglial programmed cell removal (PrCR). The absence of microglial activation on P30 (J) after IVTA is contrasted with no treatment on P14 (K).



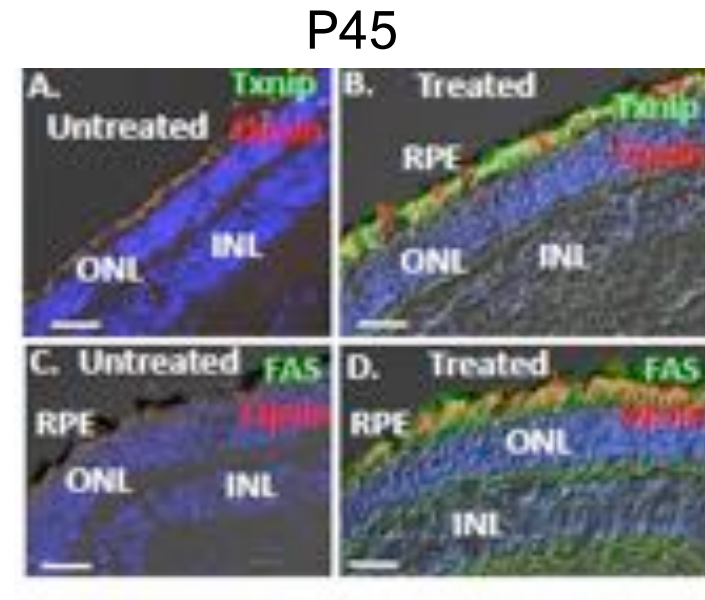
Preservation of rods allows IVTA to maintain cone OS

Representative immunostaining for cone opsin in the central and mid-peripheral retina showing identification of cone OS after IVTA at P65 (B,B'), but no cone OS after sham injection (C,C').



IVTA maintains expression of glucose-dependent genes in the ONL

Txnip protein and FAS enzyme are important in aerobic glycolysis and OS synthesis. They are induced in IS, along with cone opsin in OS, after IVTA (B,D), but not after sham injection (A,C).



Conclusions

Chronic ER stress resulting from mutant misfolded protein in rods initiates activation of retinal microglia and removal of rods in RP. IVTA, a slow release intravitreal glucocorticoid, inhibits microglia by expression of a checkpoint inhibitor on rods, with surviving rods promoting glucose transport to cones for at least 60 days post-injection. The efficacy of an intravitreal glucocorticoid implant in achieving a similar effect is being studied.