Modulating ocular hypertension induced accelerated aging in rodent retina.

UCI Center for Translational Vision Research

UCI Gavin Herbert Eye Institute

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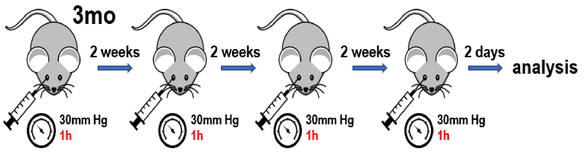
Introduction

Glaucoma is the leading cause of irreversible blindness worldwide. Increased intraocular pressure and age are the most important and consistent risk factors. Currently, lowering the IOP is the only clinically proven therapeutic paradigm. However, many glaucoma patients continue to progress in the disease despite achieving optimal IOP levels under IOP-lowering therapy. It is necessary to bridge a gap in the current state of knowledge on the pathophysiology of glaucoma to enable more successful therapeutic approaches. Addressing the relationship between the two most important risk factors, age and IOP, is thus critical in elucidating the molecular mechanisms of glaucomatous neuropathy.

Long-term IOP fluctuation has been reported to be a strong predictor for glaucoma progression, especially in individuals with low mean IOP levels. Given the role of IOP fluctuation and its dysregulation in glaucoma patients, it is critical to study the disease characteristics in the models which recapitulate the repetitive character of IOP elevation in glaucoma.

We therefore researched the dynamic of changes employing repeated IOP model. We next explored senolytic drugs which remove senescent cells as a potential therapeutic avenue to attenuate the progression of visual decline upon repetitive stress.

Methods



Visual function: 1) RGC activity: was assessed with the recording of pattern ERG (PERG). 2) V1 potentials were recorded by the means of visually evoked potentials (VEP).

RGC number: was quantified using immunofluorescence with anti-RBPMS antibody of flat-mounted retinas.

Molecular analysis: For molecular analysis, the expression of the selected group of genes was measured using RT-qPCR.

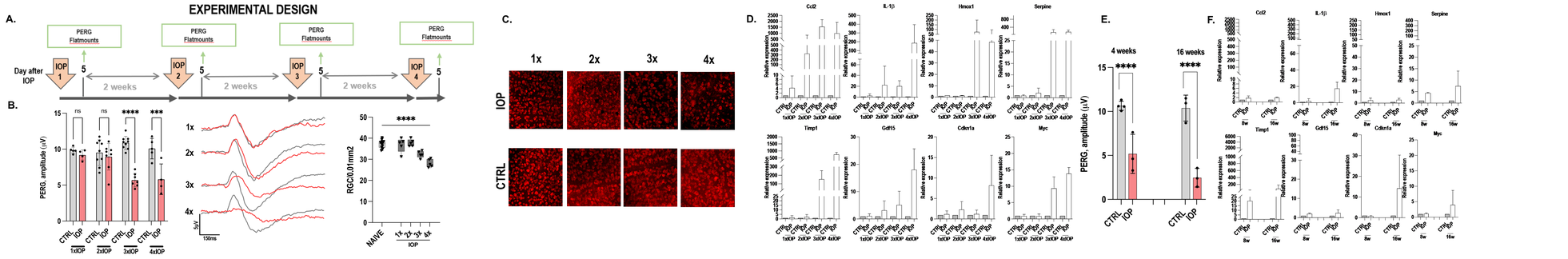


Figure 1: Structural and molecular changes upon exposure to elevated intraocular pressure are persistent. ***A.*** *Experimental design* ***B.*** *3-month-old animals exposed to the repeated elevation of the IOP exhibit progressive decline in RGC function and structure.* ***C.*** *RBPMS immunostained retinas show progressive RGC loss in response to elevated IOP.* ***D.*** *Retinas exposed to repeated IOP model show progressive upregulation of markers associated with aging, senescence and inflammation.* ***E.*** *Biology of the animals was followed for 4 and 16 weeks after final IOP. The PERG revealed reduced amplitudes at 4 and 16 weeks following exposure to elevated IOP.* ***F.*** *Gene expression analysis at 4 and 16 weeks following exposure to stress revealed persistent upregulation of genes.*

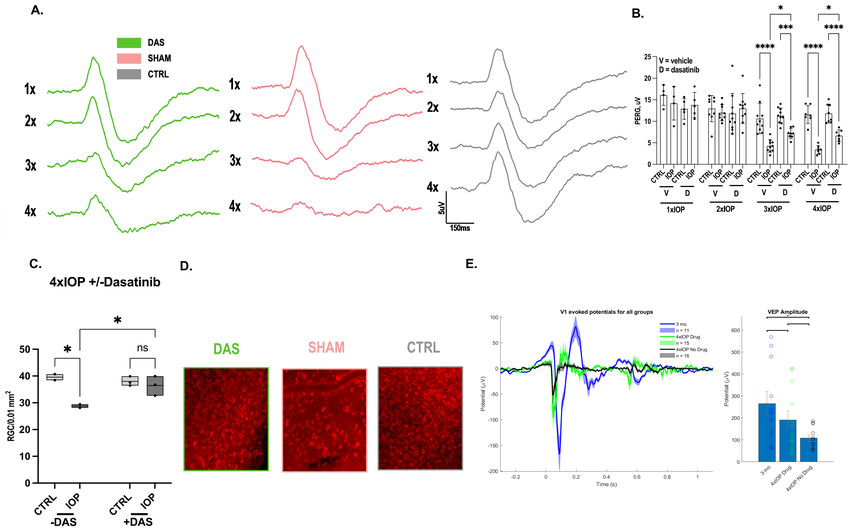


Figure 2. **Senolytic drug intervention between stress instances can protect retinal ganglion cells from death and prevent subsequent vision loss.** *3-month-old mice were exposed to 4 events of elevated intraocular pressure and then treated with Dasatinib i.p. on the day of IOP and 4 days after.* ***A.*** *Dasatinib i.p. (green) prevents RGC function loss in when compared to sham treated animals (red). Non-IOP treated animals shown for control (grey).* ***B.*** *PERG analysis shows statistically significant improvement for animals dasatinib treated animals exposed to 3 and 4 events of elevated IOP.* ***C.*** *Dasatinib i.p. treatment prevents RGC loss in animals exposed to 4xIOP.* ***D.*** *Whole retina flat-mounts show protective effect of dasatinib.* ***E.*** *Dasatinib treatment improves V1 potentials in 4xIOP treated animals.*

Results

Following each instance of IOP elevation, animals underwent a visual function assessment by means of pattern PERG which revealed progressive loss of RGC function in response to repeated IOP. Structure assessed by RGC counts on flat-mounted retinas stained with anti-RBPMS antibodies has shown a progressive loss of RGC in response to IOP elevation. Gene expression differences between IOP-treated and controlled eyes were assessed by qPCR and revealed progressive upregulation of genes associated with aging, senescence and inflammation. Wfound that visual function was significantly reduced at both 4 and 16 weeks after the fourth IOP event. Moreover, expression of genes associated with aging and senescence measured at 8 and 16 weeks after the final IOP event, revealed that a difference persisted between the IOP and control groups at both time points. Next, we sought to establish whether senolytic treatment (Dasatinib i.p.) can prevent functional and structural decline. Drug administration was continued for four consecutive days after the IOP elevation event, as previously done. Interestingly the i.p. treatment with dasatinib significantly improved the RGC function in 3xIOP and 4xIOP treated mice in comparison to i.p. sham treated animals. The functional improvement was also evident in PERG analysis which showed statistically significant improvement in 3x and 4x groups ($p<0.05$). To explore the impact of senolytic treatment on structure we performed whole retinal flatmounts and stained retinas with anti-RBPMS anitibodies to compare RGC numbers in dasatinib and sham-treated retinas. Our data shows that dasatinib treated animals that underwent 4 events of IOP elevation had significantly higher RGC numbers than sham treated animals, thus confirming a protective effect of senolytic approach. Representative images of whole retina flat-mounts are shown. Next, evaluated the integrity of the visual pathway by recording visually evoked potentials (VEP) in both dasatinib and sham treated animals following 4 events of IOP elevation. Our results show statistically significant improvement in responses recorded in the primary visual cortex in dasatinib treated animals in comparison to sham treated animals exposed to 4 instances of IOP increase. In summary, our data confirmed our initial hypothesis that dasatinib can impede visual decline upon repetitive IOP elevation and prevents RGC loss in the young retina.

Summary

Our study has revealed that chronic stress can expedite the aging of tissues, leading to a progressive decline in vision. Furthermore, we have shown that resulting vision loss and gene dysregulation is long lasting. In our project we sought to modulate stress induced aging by targeted removal of senescent cells. Senescent cells accumulate due in aging and disease while resisting programmed cell death. They pose a threat by persisting and harming neighboring tissues. Our findings demonstrate that targeted elimination of senescent cells can offer protection in an animal model of glaucoma, averting the loss of retinal ganglion cells, which are predominantly affected neurons in glaucoma in humans.

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