Retinal Gap Junctions Form Novel Targets for Neuroprotective Therapy for Glaucoma

Stewart Bloomfield, Sandeep Kumar, Alexandra Benavente-Perez, Reynolds Ablordeppey, Carol Lin, Suresh Viswananthan, Abram Akopian
Graduate Center for Vison Research, SUNY College of Optometry, New York, NY 10036 USA
Shaffer Grant, Glaucoma Research Foundation

STATE UNIVERSITY OF NEW YORK
COLLEGE OF OPTOMETRY

Introduction

We have reported that blockade or genetic deletion of retinal gap junctions (GJs) prior to or after induction of elevated intraocular pressure (IOP) offers significant protection of retinal and optic nerve structure and function in a mouse model of glaucoma (*Akopian et al., 2017*). These data suggest that neuronal GJs in retina are potential targets for neuroprotective therapy for glaucoma patients. However, data from rodents often do not translate well to humans. We therefore needed to determine whether the neuroprotective actions of GJ blockade in the mouse could be recapitulated in the primate. To accomplish this, we have created a new model of experimental glaucoma in the marmoset, a New World primate. The model is based on the same intracameral injections of polystyrene microbeads used in the mouse to raise IOP leading to an experimental glaucoma phenotype.

Design and Methods

Experimental glaucoma was induced in male and female young adult (12-14 months old) marmosets by IOP elevation induced by intracameral injections of 10 µm-diameter polystyrene microbeads. The intracameral injections were made unilaterally with 20-25 µL of microbead suspension (~14.4 x 10^7 beads) and then repeated at week 4. An equivalent volume of phosphate-buffered saline (PBS) was injected in contralateral eyes to serve as sham controls. IOP measurements were made weekly for up to 10 weeks after the microbead injection between 10 AM and 12 PM, to minimize the effect of diurnal IOP variations. For GJ blockade, meclofenamic acid (MFA) was delivered intravitreally (25 µL of 2.5 mM solution) at weekly intervals for the 10-week protocol. At 10 weeks after initial microbead injection, structural and functional changes were evaluated by cell counts in the GCL, measure of active gliosis by GFAP immunolabeling, counts of astrocytes and axons in the optic nerve, OCT measures, and ERG recordings. Comparisons were made between control, bead-injected and bead injected + MFA eyes. All data are presented as mean +/- SEM. Statistical significance was determined using two-tailed Student's t-test. Values of P < 0.05 were considered statistically significant.

Results

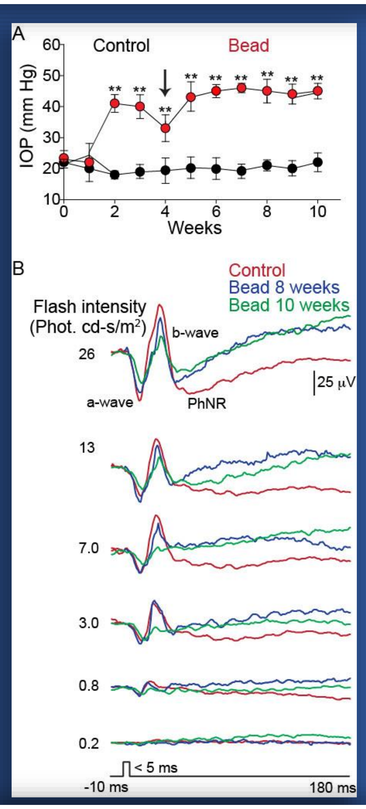

Figure 1. Effects of IOP elevation generated by microbead injection on the flash ERG. (A) Data showing sustained IOP elevation for a 10-week period, beginning at 2 weeks after an initial microbead injection followed by a second injection at week 4 (arrow) compared to control (sham injection of PBS) eyes. **P < 0.01. (B) Full field photopic flash ERG recordings from an individual marmoset in response to increasing stimulus strengths at 8 weeks (blue) and 10 weeks (green) after initial microbead injection. This is compared to the control ERG recordings, which is an average of the responses from 15 age-matched control animals (red). The PhNR in microbead-injected eyes was marked reduced from control levels beginning at 8 weeks, whereas significant reduction of the a- and b-wave amplitudes was not seen until 10 weeks.

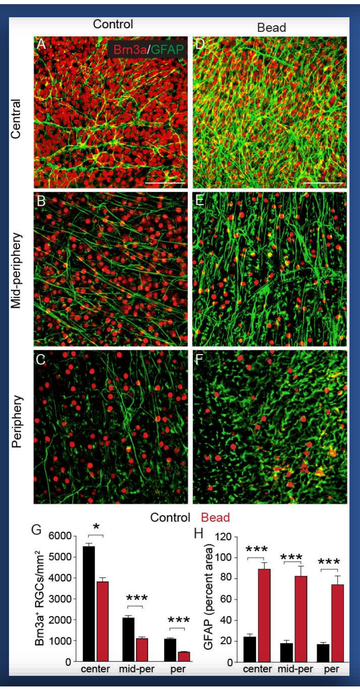

Figure 2. Territorial RGC loss and gliosis in glaucomatous marmoset eyes. (A-C) Confocal images from the central, mid-peripheral, and peripheral regions of control retinas immunostained with anti-Brn3a and anti-GFAP to visualize RGCs and astrocytes, respectfully. Scale bar = 50 µm. (D-F) Confocal images of retinas at 10 weeks after the initial microbead injection. Conventions are the same as in panels A-C. Scale bar = 50 µm. (G) Histogram showing the number of RGCs at different eccentricities in control retinas and in retinas at 10 weeks after initial microbead injection. (H) Histogram quantifying GFAP expression in control retinas and retinas at 10 weeks after initial microbead injection. Projection of 3 images, z = 1 µm for all images. For all histograms, n = 3 control retinas and n = 4 microbead-injected retinas. Data are presented as mean ± SEM. *P < 0.05, ***P < 0.001.

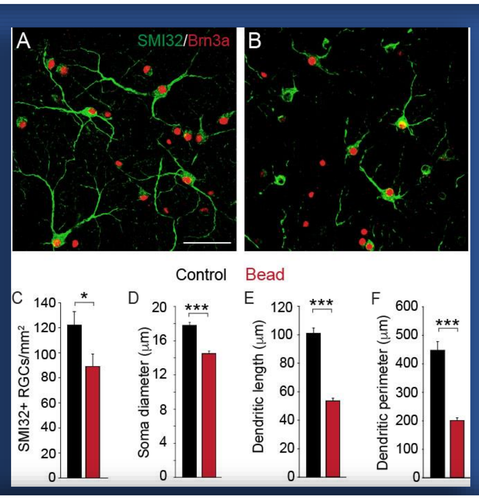

Figure 3. Effect of elevated IOP on dendritic morphology of α-RGCs. (A) Confocal image of α-RGCs in peripheral retina whose dendrites are labeled with anti-SMI32 and somata labeled with anti-Brn3a. Scale bar = 50 µm. (B) At 10 weeks after the initial microbead injection, there is a marked loss of α-RGCs with surviving cells showing reduced dendritic branching and soma shrinkage. (C) Histogram quantifying the reduced number of SMI32-positive α-RGCs at 10 weeks after initial microbead injection compared to control values. (D) Histogram comparing the mean soma size of α-RGCs in normal and glaucomatous retinas. (E, F) Histograms quantifying the reduced dendritic length and perimeter of the dendritic fields of α-RGCs in glaucomatous retinas as compared to controls. For all histograms, n = 3 control retinas and n = 4 retinas in microbead-injected eyes). Projection of 7 images, z = 1µm steps for panels A and B. Data are presented as mean ± SEM. *P < 0.05; ***P < 0.001.

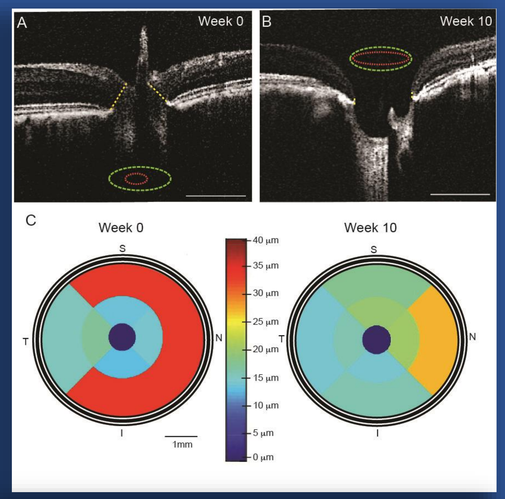

Figure 4. Microbead-injected marmoset eyes show expanded cupping of the optic nerve head and RNFL thinning characteristic of glaucoma. Representative cross-sectional OCT scan from a marmoset before (A) and 10 weeks after (B) intracameral injection of microbeads. The green outer ring and red inner ring represent the optic nerve head and cup, respectively. The cup was manually identified as the area within the minimum distance between Bruch's membrane opening (BMO) and the internal limiting membrane (BMO-minimum rim width) (yellow straight dotted lines). Scale bar = 1 mm. (C) Schematic color maps summarizing the average RNFL change occurring from baseline (week 0) to the end of treatment in glaucomatous eyes using the Iowa Reference Algorithms and plotted as color maps using Matlab (Mathworks).

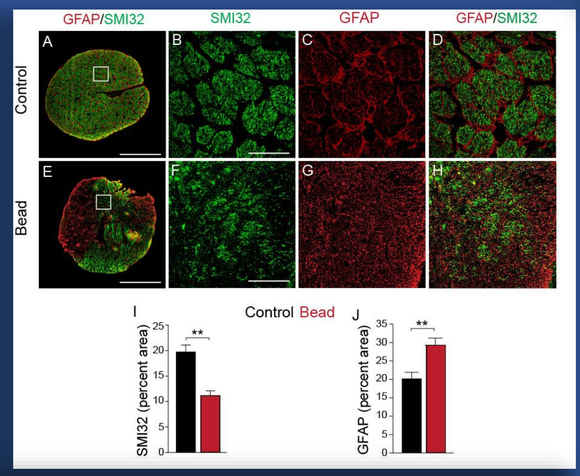

Figure 5. Effects of elevated IOP on optic nerve morphology. (A-D) Representative cross-sectional images of the lamina cribrosa region of the optic nerve from control animals immunostained for SMI32 and GFAP to visualize axonal and astrocytic structure, respectively. Rectangle in panel A shows area of higher magnification in panels B-D. Scale bars = 100 µm for panel A and 25 µm for panels B-D. (E-H) Representative cross-sectional images of the glial lamina region of the optic nerve from animals 10 weeks after initial microbead injection. A marked disruption of the SMI32-labeled axonal bundles and the honeycomb pattern of GFAP astrocytes is evident. Conventions are the same as in A-D. (I) Histogram quantifying SMI32 labeling in the cross sections of the optic nerves of marmoset under control and glaucomatous conditions (*n* = 3 control eyes and *n* = 4 microbead-injected eyes). (J) Histogram quantifying GFAP labeling in cross sections of optic nerves under control and glaucomatous conditions (*n* = 3 control eyes and *n* = 4 microbead-injected eyes). Projection of 5 images, z = 1-µm steps for all images. Data are presented as mean ± SEM. **P < 0.05.

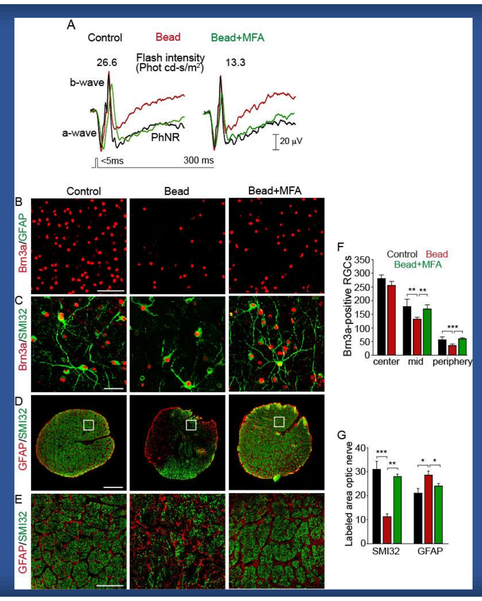

Figure 6. Blockade of GJs offers significant neuroprotection in the glaucomatous marmoset eye. (A) The amplitude of the photopic negative response (PhNR) was significantly reduced in glaucomatous eyes of untreated but not MFA-treated animals. (B) Confocal images of the peripheral marmoset retina immunostained for Brn3a at week 0 (Control), and 10 weeks after the initial microbead injection withor without MFA treatment. Bar=100 µm. (C) SMI32-positive α-RGCs in glaucoma- tous retinas show significant changes in dendritic structure, which was prevented by MFA treatment. Bar=50 µm. (D) Representative cross-sectional images of the optic nerve head from control and glaucomatous animals with or without MFA treatment immunostained for GFAP and SMI32. Bar = 500 µm. (E) Higher magnification of areas in D show the immunolabeling pattern for GFAP and SMI32 with and with- out MFA. Bar = 100 µm. (F) Quantification of RGC loss at 10 weeks with or without MFA. (G) Quantification of GFAP and SMI32 labeling in the cross sections of the optic nerve head under control and glaucomatous conditions with or without MFA treatment ***P < 0.001, **P <0 .01, *P < 0.05 (n=3 retinas and 3 optic nerves).

Conclusions

1. We have established a model of experimental glaucoma in the marmoset following the microbead occlusion technique applied in other animals, including the mouse. The model shows established criteria of experimental glaucoma, including: (i) elevated IOP for at least 10 weeks; (ii) a significant reduction of the PhNR of the ERG; (iii) a 25-65% loss of RGCs dependent on retinal eccentricity; (iv) retraction of dendrites in surviving RGCs; (v) thinning of the RNFL, and (vi) loss of astrocytes and axons in the optic nerve head.

2. Our pilot data from four marmosets suggests that blockade of retinal GJs by invitreal application of MFA does in fact provide significant protection of retina and optic nerve structure and function in glaucomatous eyes. These preliminary data reinforce our hypothesis that retinal GJs are novel targets for neuroprotective therapy to prevent progressive cell loss and visual dysfunction associated with glaucoma.

Next Steps

The glaucoma model in the marmoset described here forms a robust method to study the disease etiology, progression, and potential therapies in the non-human primate, allowing for more effective translation of animal data to humans.

The next step will be to continue experiments to determine whether pharmacological blockade of blockade of retinal GJs prior to or after induction of elevated intraocular pressure (IOP) with microbeads offers significant protection of retinal and optic nerve structure and function in the marmoset as we have shown in a mouse model of glaucoma (*Akopian et al., 2017*).

Acknowledgements

This study was funded by a Shaffer Grant of the Glaucoma Research Foundation and NIH grants EY026024 and 007360.

References

Akopian , Kumar S, Ramakrishnan H, Roy K, Viswanathan S, Bloomfield SA (2017) Targeting neuronal gap junctions in mouse offers neuroprotection in glaucoma. J. Clin. Invest. 127:2647-2661.