Neuroprotection through altered scleral biomechanics

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Abstract

Vision loss from glaucoma is due to death of retinal ganglion cells (RGC) – nerve cells of the eye that project axons to the brain. Although, the mechanisms of RGC death are complex and multifactorial, remodeling of the tissue that surrounds the exit of RGC axons from the eye (the optic nerve head, peripapillary sclera, and lamina cribrosa) is a hallmark of glaucoma and an early event in glaucoma development. Previously, we showed that systemic treatment with the angiotensin II type 1 receptor blocker (ARB1) losartan prevented RGC loss in a mouse model of IOP glaucoma by targeting remodeling processes that occur in the sclera during glaucoma[1]. This experiment served as a proof-of-principle that neuroprotection could be accomplished by targeting glaucomatous scleral remodeling. This experiment also provided a valuable lead for future investigations into scleral remodeling using pharmacologic tools. Here we have used cell culture of primary, human scleral fibroblasts to show that ARB1 compounds inhibit fibroblast to myofibroblast transdifferentiation by inhibiting SMAD phosphorylation. In addition, we developed biodegradable microparticles for sustained and local delivery of the ARB1 irbesartan. Taken together these results offer a foundation to investigate the role of fibroblast transdifferentiation in scleral remodeling and develop therapeutics to target this process and stop glaucomatous vision loss.

Advantages of targeting fibroblasts

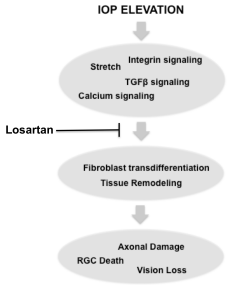
- Targets the early stages of glaucoma damage
- Does not require intraocular delivery or penetration through the blood retina barrier

Methods

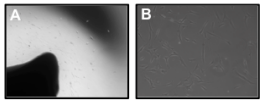
- Primary cell lines from peripapillary sclera were created
- Transdifferentiation to a myofibroblast phenotype was induced by TGFβ treatment
- Myofibroblast differentiation was assessed by αSMA immunoblot and collagen contraction assay
- Microparticles for sustained release of the ARB1 blocker irbesartan were developed
- Irbesartan microparticles were tested in experimental mouse glaucoma

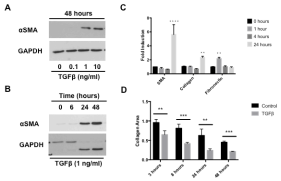
Results

(1) *IOP elevation causes scleral remodeling, axonal damage, and RGC loss.*

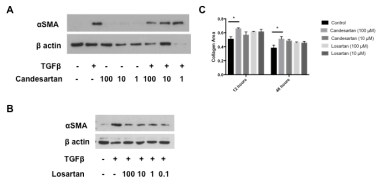


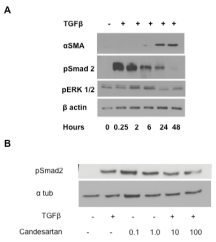
(2) *Scleral fibroblasts undergo myofibroblast trans-differentiation following TGFβ exposure*



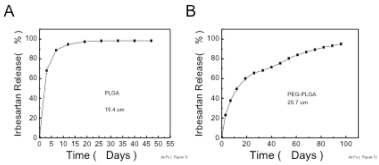


(3) *ARB1 pretreatment prevents fibroblast to myofibroblast transdifferentiation*





(4) *Microparticles for sustained release of ARB1 inhibitors*



	Mean Peak IOP (STDEV)	Mean Total Integral (STDEV)	Positive Integral (STDEV)	Axonal Loss (STDEV)
Control Injection	31.74mmHg (5.0)	216.04 mmHG days (186.7)	229 mmHG days (161.41)	48.6% (30.16)
Irbesartan Microparticles	34.96 mmHg (8.2)	102.11 mmHG days (174.6)	139.07 mmHG days (133.42)	35.17% (35.17)
p value (t-test)	0.11	0.03	0.03	0.16

Conclusions

1. *Scleral fibroblasts undergo myofibroblast transdifferentiation following TGFβ exposure*
2. *Transdifferentiation is inhibited by ARB1 pretreatment*
3. *pSMAD2 is inhibited by ARB1 pretreatment*
4. *Microparticles achieve sustained release of ARB1 inhibitors over 80 days*

References & Acknowledgements

Quigley HA, Pitha IF, Welsbie DS, et al. Losartan Treatment Protects Retinal Ganglion Cells and Alters Scleral Remodeling in Experimental Glaucoma. *PLoS ONE*. 2015;10(10):e0141137. doi:10.1371/journal.pone.0141137.

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