An Improved Circumlimbal Suture Model That Exhibits Gradual Increase in Intraocular Pressure

Mathew DJ[1,2,3,4], Livne-Bar I[1,2,3,4], Sivak JM[1,2,3,4]

[1]Department of Ophthalmology and Vision Sciences, University of Toronto, Toronto, Canada; [2]Donald K. Johnson Eye Institute, Toronto, Canada; [3]Krembil Research Institute, Toronto, Canada; [4]University Health Network, Toronto, Canada

Introduction

- Chronic ocular hypertension animal models suffer from limitations, such as inflammation, high initial intraocular pressure (IOP) spike, ischemia, short duration of elevated IOP, and risk of infection and hemorrhage.
- A better model is needed to provide a more accurate means of evaluating neuroprotection strategies and ocular hypotensive drugs.
- A model with prolonged elevated IOP (2-3 months) without intraocular entry may be more relevant, with reduced risks of inflammation and infection.

Purpose

- To characterize a rodent model of gradual chronic ocular hypertension, without an initial intraocular pressure (IOP) spike common to many current inducible models

Methods

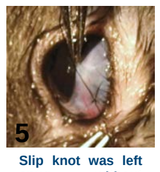
- Six-week-old male Long Evans rats used
- Intraperitoneal Ketamine-Xylazine cocktail anesthesia

Tip of Tonolab rebound tonometer, iCare, directed perpendicular to the centre of the cornea, at an appropriate distance

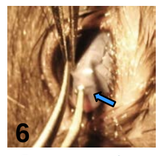
Subconjunctival bites taken with 8-0 Nylon 1-1.5 mm posterior to the limbus

After 5-6 subconjunctival suture passes, the suture emerges close to the initial subconjunctival pass (white arrows)

Slip knot (granny knot) denoted by white arrow



Slip knot was left snug without tightening and secured with three additional simple knots

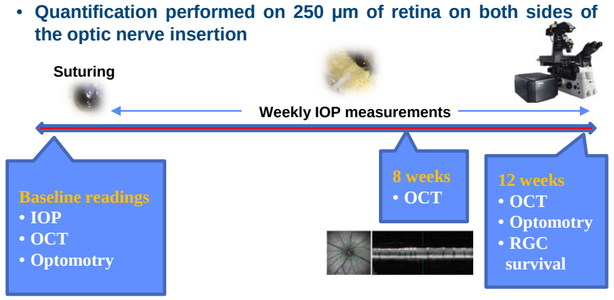


One arm of a forceps could be easily passed under the suture (blue arrow)

- Care taken to make sutures snug, without inducing an IOP spike, generating gradual ocular hypertension (gOHT) as the sutures tightened over time
- Control eyes (CON) loosely sutured
- IOP measured using Tonolab rebound tonometer (iCare, Helsinki, Finland) before the procedure, immediately after securing the slip knot and weekly thereafter

Methods (Cont'd)

- Eyes collected after 12 weeks of elevated IOP
- Following cryosectioning, confocal microscopy used to evaluate cell survival in the retinal ganglion cell (RGC) layer (RBPMS) and glial cell reactivity (GFAP)
- Quantification performed on 250 µm of retina on both sides of the optic nerve insertion

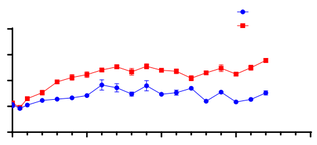


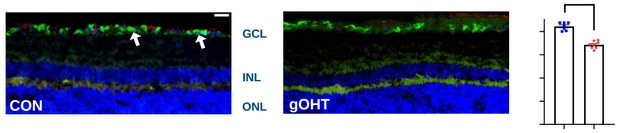
Results

	CON	gOHT	P value
n	6	12	
IOP (mmHg)			
Baseline	10.2±0.2	10.5±0.3	0.33
Post-suturing	9.2±0.2	9.6±0.2	0.12
12 weeks	15.2±0.8	27.8±0.8	<0.0001

Intraocular pressure recordings at baseline, immediately after suturing, and at 12 weeks of elevated IOP

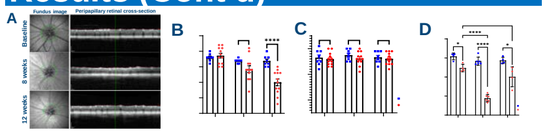
Line graph showing the intraocular pressure trend over time in CON and gOHT. * indicates time points at which the difference in IOP between CON and gOHT were statistically significant (p<0.001).



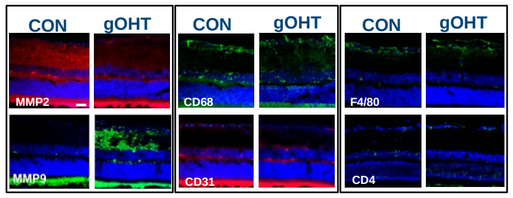


Retinal images from CON and gOHT eyes stained for RBPMS (green, arrows) and GFAP (red), in addition to the nuclear marker DAPI (blue) (scale bar indicates 50 µm). Quantification of RGCs indicates significantly decreased survival in gOHT compared to CON (****p<0.0001, bars are SE). Scale bar indicates 50 µm. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer

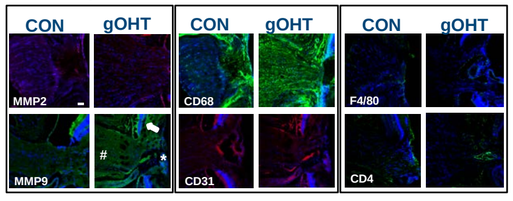
Results (Cont'd)



The gOHT model results in progressive RNFL thinning and decreased vision at 12 weeks. (A) Representative fundus and corresponding cross-sectional OCT images (B) Progressive thinning in the gOHT group compared to CON highly significant at 12 weeks. (C) Outer retinal thickness did not show statistically significant change at any time point. (D) Visual acuity at 12 weeks shows a highly significant difference. However, there was a statistically significant decrease in vision in all groups post-suturing (p<0.05). Notably, animals that responded poorly in terms of IOP elevation post-suturing did not show a statistically significant difference from CON. ****p<0.0001, *p<0.05, bars are SE; RNFL, retinal nerve fiber layer.

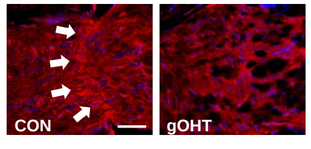


Representative immunostaining panels of CON and gOHT retinas. Compared to CON eyes, MMP2 appeared decreased and MMP9 is increased in the gOHT inner retina. There was no notable difference in staining for CD68, CD31, F4/80, or CD4. Scale bar indicates 50 µm.

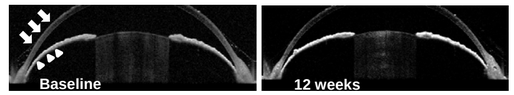


Chronic gOHT results in optic nerve head tissue remodeling and neuroinflammation. Representative immunostaining panels of CON and gOHT in sections of optic nerve head tissue. There was mildly increased staining for MMP9 in gOHT compared to CON, and CD68 staining was strongly increased. There were no notable differences for any other marker (scale bar indicates 50 µm; for orientation, the white arrow indicates DAPI stained retinal layers, * indicates the vitreous cavity adjacent to optic nerve head, and # indicates the optic nerve).

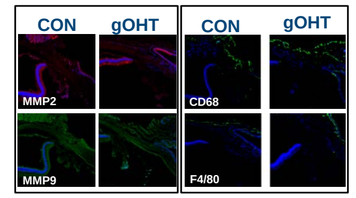
Representative phalloidin staining (red) highlights an intact pseudolaminar region in CON tissue (arrows), which was disrupted with neuronal loss in gOHT eyes (scale bar indicates 50 µm).



Results (Cont'd)



OCT images of the anterior chamber angle show in the same eye the area between the peripheral cornea (arrows) and iris (arrowheads). The angle remained open until the last follow-up after 12 weeks of elevated IOP, indicating that this was an open angle glaucoma model.



No significant differences in staining between CON and gOHT angles for MMP2, MMP9, CD68 and F4/80

Conclusions

- The gOHT model produces chronic mildly elevated IOP in rats, accompanied by loss of retinal ganglion cells and visual function, and no evidence of inflammatory cell infiltration.
- The advantages with this model include the absence of a pathological initial spike in IOP, no intraocular entry or inflammation, and induction of a gradual increase in IOP, similar to clinical glaucoma.

References

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