Glucagon-like Peptide 1 Receptors on Myeloid Cells are Necessary for GLP1R-mediated Retinal Ganglion Cell Protection in Glaucoma

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Purpose

- Glaucoma is a degenerative disease of retinal ganglion cells (RGCs) that is often associated with increased intraocular pressure.
- Previously, we have shown that glucagon-like peptide 1 receptor (GLP1R) agonists, a class of medication used in diabetic care and weight loss, promote RGC survival in a mouse model of hypertensive glaucoma.[1-2]
- GLP1R has been found on retinal macrophage/microglia (M/M; **Fig 1**).
- It is unknown whether M/M receptor expression is necessary to promote RGC survival in ocular hypertension.
- **We used a mouse model of hypertensive glaucoma with inducible M/M-specific GLP1R knockout (*GLP1RmmKO*) to answer this question.**

Methods

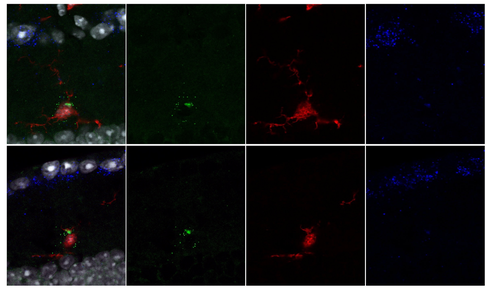
- A strain of *GLP1RmmKO* mice was generated by crossing *Cx3cr1*$^{(CreER)}$ (JAX #020940) with *GLP1R*$^{(fl/fl)}$ (**Fig 2A**).
- At 8 weeks of age, *GLP1RmmKO* mice received either IP tamoxifen (*mmKO-T*) 5mg over 5 days (20mg/ml in corn oil) to induce knock out (**Fig 2A**), or IP corn oil (*mmKO-CO*) as controls.
- After 1-week, retinal M/M cells (11b+) from C57BL/6J (WT), *mmKO-CO* and *mmKO-T* were sorted using FACS and their GLP1R expression were analyzed using qPCR.
- One week after induction, magnetic microbeads were injected into the anterior chamber of one eye while the fellow eye received an injection of balanced salt solution (BSS).
- Intraocular pressure (IOP) was measured immediately before the first injection and weekly thereafter using iCare TONOLAB tonometer. Eyes with IOP < 21mmHg at week 2 received a 2nd bead injection at week 3.
- For 6 weeks, treatment groups received daily SQ injections of liraglutide (Lir) at 400 µg/kg, a long-acting GLP1R agonist, while controls received equal volume PBS injections.
- Retina flat mounts were immunolabeled for Rbpms and Iba1 to quantify RGC and myeloid density (per 40x HPF), respectively. RGC axon counts were quantified using ultrathin (700nm) optic nerve cross-sections.

Results

- Compared to BSS-injected controls, WT mice injected with beads demonstrated increased GLP1R expression in 11b+ cells following IOP elevation (p=0.004). A similar trend was seen in *mmKO-CO* mice (p=0.005). In comparison, *mmKO-T* mice did not demonstrate an increase in GLP1R expression in bead-injected eyes (p=0.69) (**Fig 2B**).
- Compared to BSS-injected controls, GLP1R expression in 11b- cells demonstrated a non-significant but a trend towards an increase following IOP elevation in WT, *mmKO-CO* and *mmKO-T* mice (**Fig 2C**).
- IOP remained elevated for 6 weeks after bead injections (p<0.001 compared to BSS-injected eyes) (**Fig 3**).
- In the liraglutide-treated groups, *mmKO-CO* mice demonstrated near-complete rescue of RGCs (p=0.11) while *mmKO-T* mice demonstrated incomplete rescue of RGCs (p=0.01) in bead vs. BSS-injected eyes. In the PBS-treated groups, both *mmKO-CO* and *mmKO-T mice* demonstrated similar RGC loss (p<0.001) in bead vs. BSS-injected eyes (**Fig 4A&C**).
- Compared to BSS-treated eye, liraglutide-treated *mmKO-T* mice demonstrated 14.9% (p=0.04) RGC density reduction in the bead-injected eyes. Whereas PBS-injected *mmKO-CO and mmKO-T mice* showed 29.3% (p<0.0001) and 29.5% (p<0.0001) RGC density reduction in the bead-injected eyes. Liraglutide-treated *mmKO-T* mice demonstrated partial and significant rescue when compared to PBS-treated *mmKO-CO* (p=0.01) *and mmKO-T* (p=0.002) mice (**Fig 4B**).
- In the liraglutide treated groups, preliminary axon analysis demonstrated near-complete axon rescue (p=0.60) in *mmKO-CO* mice and incomplete rescue (p=0.02) in *mmKO-T* mice. In the PBS-treated groups, both *mmKO-CO* and *mmKO-T mice* demonstrated significant axon loss (p=0.005 & p=0.0003) in bead vs. BSS-injected eyes (**Fig 5A&C**).
- Compared to BSS-treated eyes, liraglutide-treated *mmKO-T* mice demonstrated 34.0% (p=0.07) axon loss in the bead-injected eye. Whereas PBS-injected *mmKO-CO and mmKO-T mice* showed 51.27% (p=0.007) and 50.02% (p=0.001) axon loss **(Fig 5B)**.

Results (continued)

Figure 1. GLP1R expression is present on retinal macrophage/microglia in ocular hypertension.



C57BL6/J mice were injected with microbeads to increase IOP for 6 weeks. Retinal cryosections were probed for *GLP1R* (green) and the RGC marker *Rbpms* (blue) using *in situ*. Myeloid cells were labeled with antibodies for *Iba1* (red). Nuclei were labeled with DAPI (gray).

Figure 2. Generation of *Cx3cr1*$^{(CreER)}$ specific GLP1R knockouts with tamoxifen (Tam) induction (A). GLP1R expression in 11b+ (B) and 11b- (C) sub-populations of retinal cells in C57B/6J (WT) and *GLP1RmmKO* treated with corn oil (*mmKO-CO*) or tamoxifen (*mmKO-T*).

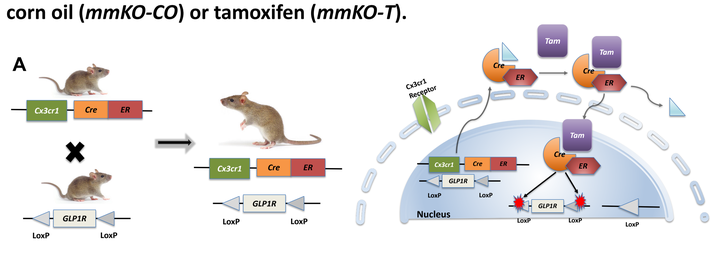


Figure 3. IOPs in microbead (bead) and BSS-injected eyes of *Cx3cr1*$^{(CreER)}$ specific GLP1R knockout induced with tamoxifen (*mmKO-T*) or corn oil as controls (*mmKO-CO*). Treated with either liraglutide (Lir) or PBS injections.

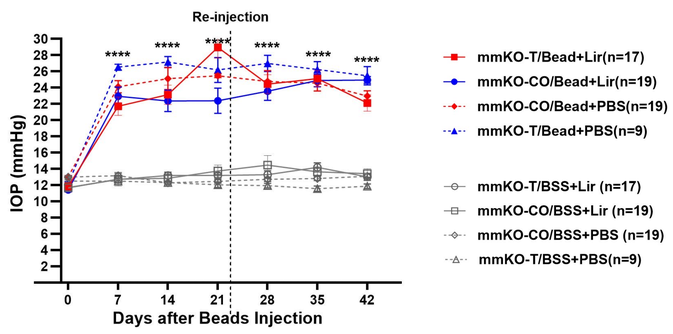


Figure 4. RGC quantification (A), relative survival percentage (B) and Rbpms labeled retinal flat mount images (C) in microbead (bead) vs. BSS- injected eyes in liraglutide (Lir) and PBS treated groups after 6 weeks .

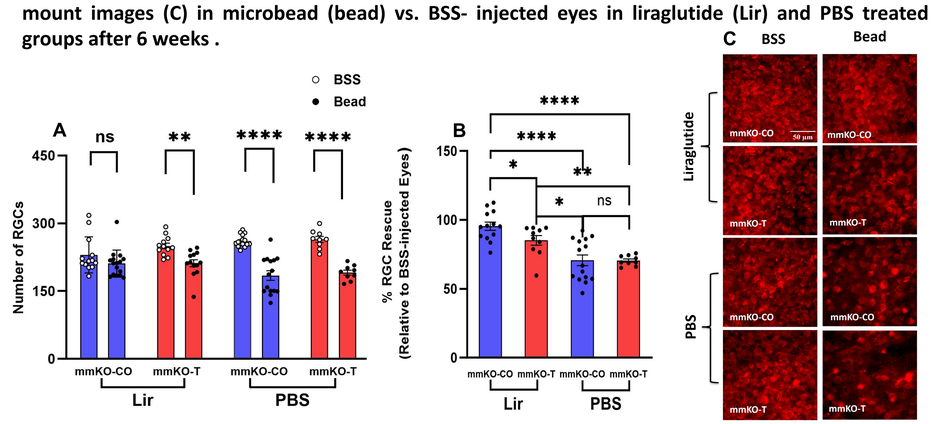
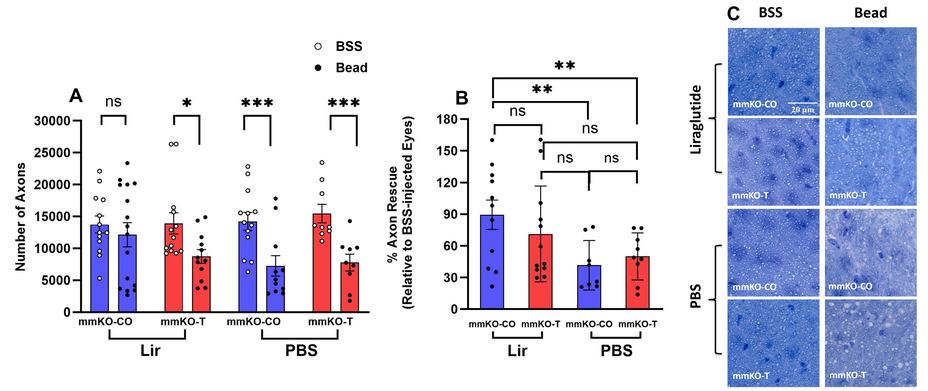


Figure 5. Axon quantification (A), relative survival percentage (B) and optic nerve cross-sections (C) in microbead (bead) vs. BSS- injected eyes in liraglutide (Lir) and PBS treated groups after 6 weeks .



Conclusions

1. Without tamoxifen induced knockout of GLP1R, treatment with liraglutide resulted in near-complete retinal ganglion cell rescue, suggesting GLP1 receptor expression on macrophage/microglia is *necessary* for promoting RGC survival in ocular hypertension.
2. In comparison, following induction of GLP1R knockout, treatment with liraglutide resulted in partial retinal ganglion cell rescue, suggesting GLP1 receptor expression on macrophage/microglia is *necessary* but *not sufficient* for promoting RGC survival.
3. Our preliminary axon analysis suggests rescue trends in line with RGC results.

References

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2. Lawrence ECN, Guo M, Schwartz TD, Wu J, Lu J, Nikonov S, Sterling JK, Cui QN. Topical and systemic GLP-1R agonist administration both rescue retinal ganglion cells in hypertensive glaucoma. Front Cell Neurosci. 2023 Jun 9;17:1156829. doi: 10.3389/fncel.2023.1156829. PMID: 37362000; PMCID: PMC10288152.

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