Insulin Resistance and Aquaporin-4 Dysregulation in Glaucomatous Damage: A Cellular and In Vivo Investigation

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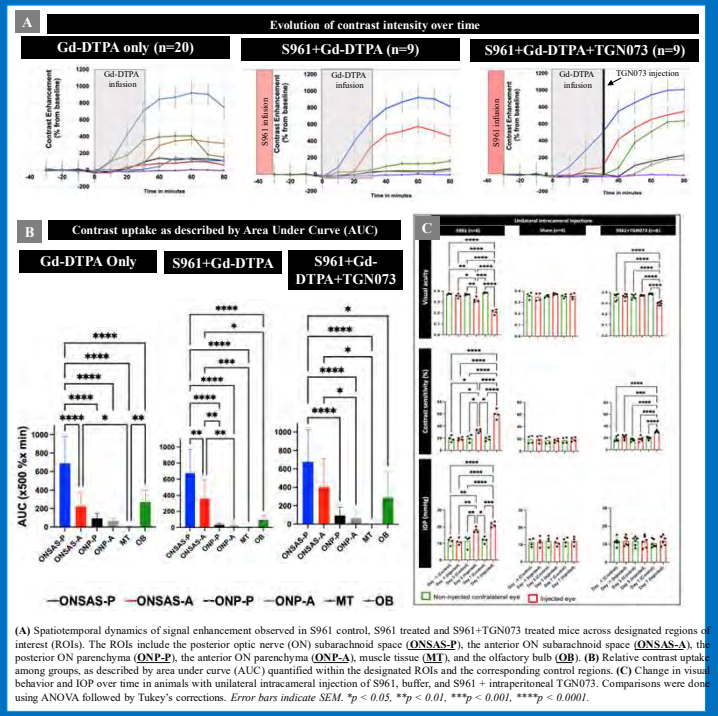
INTRODUCTION

Glaucoma is a leading cause of irreversible blindness, often associated with elevated intraocular pressure (IOP) and retinal ganglion cell (RGC) degeneration. Recent research suggests that insulin resistance may exacerbate ocular damage via aquaporin-4 (AQP4) water channel dysfunction. This study investigates if insulin resistance impairs AQP4 function in the eye, disrupts cerebrospinal fluid (CSF) dynamics in the optic nerve, and thereby, contributing to glaucomatous damage.

DESIGN & METHODS

This study employs both *in vitro* and *in vivo* models to test the central hypothesis that insulin resistance facilitates glaucomatous damage via AQP4-mediated impairment in the eye and CSF dynamics. **In Aim 1,** primary cultures of trabecular meshwork (TM) cells, ciliary body (CB) cells, and retinal ganglion cells (RGCs) were exposed to S961, an insulin resistance inducer, to assess gene and protein alterations associated with AQP4 dysregulation. Microarray analysis identified key dysregulated genes, with subsequent validation through real-time PCR and Western blotting. **Aim 2** investigates the effects of S961-induced insulin resistance *in vivo*, focusing on IOP elevation, optic nerve CSF dynamics, and visual behavior. IOP and visual function were monitored longitudinally, and dynamic contrast-enhanced MRI with the passive Gd-DTPA contrast agent was used to examine fluid dynamics in the optic nerve. Additionally, bidirectional modulation of AQP4 was tested using TGN020 (AQP4 inhibitor) and TGN073 (AQP4 enhancer) to evaluate potential therapeutic avenues for reversing insulin resistance-induced ocular dysfunction. Data were analyzed to identify gene expression patterns, functional changes in ocular tissues, CSF dynamics in optic nerve, and the impact on IOP and visual behavior including visual acuity and contrast sensitivity.

DIAGRAM



(A) Spatiotemporal dynamics of signal enhancement observed in S961 control, S961 treated and S961+TGN073 treated mice across designated regions of interest (ROIs). The ROIs include the posterior optic nerve (ON) subarachnoid space (**ONSAS-P**), the anterior ON subarachnoid space (**ONSAS-A**), the posterior ON parenchyma (**ONP-P**), the anterior ON parenchyma (**ONP-A**), muscle tissue (**MT**), and the olfactory bulb (**OB**). **(B)** Relative contrast uptake among groups, as described by area under curve (AUC) quantified within the designated ROIs and the corresponding control regions. **(C)** Change in visual behavior and IOP over time in animals with unilateral intracameral injection of S961, buffer, and S961 + intraperitoneal TGN073. Comparisons were done using ANOVA followed by Tukey's corrections. *Error bars indicate SEM.* $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

RESULTS

- ✓ S961 treatment resulted in significant gene expression changes in RGCs, CB, and TM cells, including dysregulation of AQP4, apoptosis-related genes (e.g., BCL2, BDNF), and inflammation markers (e.g., IL6, TNFα).
- ✓ AQP4 expression was upregulated in CB cells and RGCs but decreased in TM cells following S961 exposure.
- ✓ Altered AQP4 expression in RGCs persisted up to 90 days post-S961 treatment, suggesting chronic effects of insulin resistance.
- ✓ Insulin resistance induced a significant increase in IOP and a compromise in visual behavior in S961-treated mice.
- ✓ TGN073, an AQP4 enhancer, mitigated the effects of insulin resistance, improving visual behavior and IOP regulation.
- ✓ Dynamic MRI revealed impaired fluid dynamics in the optic nerve under insulin resistance, with TGN073 treatment restoring normal contrast uptake and clearance.

CONCLUSIONS

Our findings indicate that insulin resistance leads to dysregulation of AQP4 that significantly alters ocular fluid dynamics and neurodegeneration. The upregulation of inflammatory markers and apoptosis-related genes further highlights the detrimental effects of insulin resistance on ocular health. Targeting AQP4 function through modulation offers a promising therapeutic avenue for protecting retinal and optic nerve function in glaucomatous conditions associated with insulin resistance.

NEXT STEPS

- ✓ Investigate the long-term effects of AQP4 modulation in preventing or reversing retinal ganglion cell and axon degeneration in glaucoma models.
- ✓ Explore the molecular mechanisms underlying AQP4 dysregulation in response to insulin resistance, with a focus on inflammation and apoptosis pathways.
- ✓ Conduct further *in vivo* studies to assess the therapeutic potential of TGN073 and other AQP4 modulators in chronic glaucoma models.
- ✓ Evaluate the role of AQP4 in regulating intraocular pressure and cerebrospinal fluid dynamics to develop targeted treatments for glaucoma.
- ✓ Explore the translational potential of AQP4-targeted therapies in human ocular diseases linked to insulin resistance.

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