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TITLE: Therapeutic Screening for Traumatic Optic Neuropathy

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CONTRACTING ORGANIZATION: Biomedical Engineering, The Ohio State University

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14. ABSTRACT

Traumatic optic neuropathy (TON) is a blinding condition caused by trauma to the eye, head, or optic nerve. At present, the clinical standard of care is observation: there are no surgical or therapeutic interventions which have been shown to improve outcomes. We have developed a small animal model of TON and previously characterized it with electrophysiology to determine diagnostic criteria. In the present study, we use this model and electrophysiological methods to screen several candidate therapeutic approaches to TON for efficacy. To date, we have characterized partitioning of these drugs injected into the vitreous or retrobulbar space as a means for optimizing delivery. We plan to

15. SUBJECT TERMS

NONE LISTED

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INTRODUCTION

Traumatic optic neuropathy (TON) is a common consequence of battlefield or civilian injury to the eye or head and results in blindness. No treatment is currently available, at least in part due to the lack of suitable animal models for evaluating drugs which could improve visual function after injury. We have therefore developed animal models of this injury, as well as diagnostic tests by which the extent of visual deficit can be measured. Together, these offer a powerful tool for evaluating potential treatments. In this study, we will evaluate four drugs which are intended to prevent breakdown of the retina and optic nerve tissues in the aftermath of injury. By slowing or stopping these biological processes, it may be possible to retain or regain visual function. The drugs we have selected are also FDA-approved for other diseases and may be quickly used for treatment of TON.

KEYWORDS

Traumatic optic neuropathy; neurotrauma; electroretinogram; therapeutic; diagnostic; anti-inflammatory; nerve injury modeling

ACCOMPLISHMENTS

Major Goals

	Goal/Milestone	Target Date	Date Achieved/Forecast
1	ACURO approval of animal protocols	November 2022	September 2022
2	Determine local delivery routes for each therapeutic	March 2023	September 2023
2**a**	Finalize delivery routes	March 2023	October 2023
2b	Submit scientific paper detailing delivery partitioning studies	May 2023	December 2023
3	Evaluate therapeutic efficacy	March 2024	June 2024
3a	Submission of scientific paper describing electrophysiologic findings	July 2024	July 2024
3b	Submission of scientific paper describing immunohistochemical findings	August 2024	August 2024
3c	Identification of candidate therapeutic(s) for evaluation in pre-clinical animal model of TON	August 2024	August 2024

Accomplishments

Major Task 1: ACURO approval of animal protocols

ACURO approval was achieved on 15 September 2022.

Major Task 2: Determine local delivery routes for each therapeutic

We have recently completed a pilot study and evaluated which of the candidate therapeutics meaningfully accumulates in the tissue of interest (i.e. in the optic nerve and/or retinal ganglion cells). Liquid chromatography-mass spectrometry (LC-MS) was used to evaluate the presence of each therapeutic in the retina and optic nerve following (A) intravitreal injection (IVI) and (B) retrobulbar injection (RBI). The Ohio State University Mass Spectrometry and Proteomics core facility was used to assess the presence of each therapeutic. Notably, retrobulbar administration of Anakinra was abandoned after fully anesthetized animals exhibited a marked pain reflex during RBI. This was presumed to be a result of the acidic nature of the solution. No similar response was noted upon IVI of Anakinra or administration of any other candidate therapeutic.

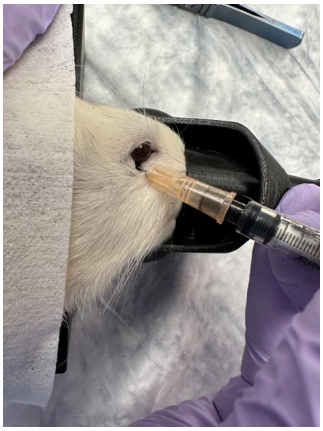

Figure 1: Retrobulbar injection of tattoo ink to demonstrate the efficacy of injection method.

Methods

Agent Administration

IVI was achieved via pars plana injection using a 28 gauge needle.

RBI has not previously been described for rats in the literature. We therefore developed a novel injection technique wherein the rat is anesthetized using a 3D-printed nose cone with isoflurane (Figure 1). A 28 gauge needle was then inserted into the ocular socket almost parallel to the bottom of the globe (9-10 o'clock). The needle was inserted until it hit the bone of the ocular socket and then pulled back slightly (<1mm). Therapeutic was then injected slowly into the ocular space. The needle was slowly removed and then the eye was held closed with light pressure for 10 seconds to let the therapeutic settle in the socket.

Agent Partitioning

Neural tissues from the retina and optic nerve were carefully isolated. Each sample was placed in a 2 mL lysing solution and weighed. Samples were extracted with 80% methanol (retina: 1:100 w/v; nerve: 1:200 w/v), then homogenized at 6800 RPM for 4 cycles (30 s per cycle with 45 s pause) using a Bertin Precellys Homogenizer. Homogenates were sonicated in a water bath for 10 minutes prior to centrifugation at 10,000 rcf for 10 min. Supernatant was injected into the LC/MS-QQQ instrument. Matrix blanks were prepared by combining the extraction from samples without any therapeutic injection.

Results

LC-MS indicated the presence of cyclosporine in appreciable quantities in the retina and optic nerve (Table 1). Both IVI and RBI resulted in measurable quantities of cyclosporine in the optic nerve. However, Ibudilast, and TUDCA were not found within the calibrated range of analysis (i.e. their concentrations were <5 ppb).

Table 1: Agent partitioning in retina and nerve

		Concentration (ppb)	
Agent	Route	Retina	Optic Nerve
Cyclosporine	IVI	8-768	<5
Cyclosporine	RBI	9-22	18-81
TUDCA	IVI	ND	ND
TUDCA	RBI	ND	ND
Ibudilast	IVI	ND	ND
Ibudilast	RBI	ND	<5
Anakinra	IVI	*	*
Anakinra	RBI	N/A	N/A
ND: Not detected			
*Proteomic mass spectrometry result pending			
N/A – abandoned as noted in the text			

Major Task 3: Evaluate therapeutic efficacy
We plan to commence these studies in October 2023. We will evaluate the efficacy of cyclosporine via both IVI and RBI routes. We may also evaluate Anakinra via IVI if it successfully reaches appreciable concentrations in the retina and/or optic nerve.

Training and Professional Development
To date, four undergraduate students and one graduate student have been trained as part of this project. Students are provided with opportunities to present at local, national, and international conferences including the Ohio State University (OSU) Neuroscience Research Institute conference, OSU Engineering in Health and Industry Research Symposium (EHIRS), OSU Chronic Brain Injury Symposium, Vision Injury Research Forum (VIRF), Biomedical Engineering Society (BMES), and Association for Research in Vision and Ophthalmology (ARVO). This has been the primary method of disseminating results prior to submission of research articles.

IMPACT

Impact on Development of the Principal Discipline(s) of the Project
We have developed a robust suite of electrophysiologic tests to fully characterize the light-adapted rat visual system. These methods have been adapted by other rodent researchers.

Impact on Other Disciplines
Nothing to report.

Impact on Technology Transfer
Nothing to report.

Impact on Society Beyond Science and Technology
Nothing to report.

CHANGES/PROBLEMS

The need to develop a novel retrobulbar injection method for rats has resulted in minor delays to the projected timeline. We do not anticipate that this will adversely affect the timeline to complete the project.

PRODUCTS

Journal Publications
Nothing to report.

Books or Other Non-Periodical, One-Time Publications

- Ryan, A.K., Rich, W.W., Jansen, P.A., Allyn, M.M., Swindle-Reilly, K.E., Reilly, M.A., Oxidative Stress in the Eye, in: *Molecular Basis of Oxidative Stress: Chemistry, Mechanisms, and Disease Pathogenesis*, 2nd edition; Frederick A. Villamena, ed., Wiley, in press.

Other Publications, Conference Papers, and Presentations

- Ryan, A.K., Landreth, C., Artis, E., Cheung, R., Rex, T.S., Racine, J., Reilly, M.A., An Electrophysiological Technique for the Diagnosis of TON in a Small Animal Model. Association for Research in Vision and Ophthalmology, Annual Meeting, New Orleans, Louisiana, April 2023.
- Ryan, A.K., Racine, J., Reilly, M.A., Diagnosing Traumatic Optic Neuropathy Using Visual Electrophysiology, Vision Injury Research Forum, Virtual, April 2023.

- Ryan, A.K., Racine, J., Reilly, M.A., An Electrophysiological Technique for the Diagnosis of TON in a Small Animal Model. Biomedical Engineering Society, Annual Meeting, San Antonio, Texas, October 2022.
- Ryan, Annie K.; Landreth, Claire; Cheung, Rachael; Artis, Elizabeth; Rex, Tonia S.; Racine, Julie; Reilly, Matthew A. "An Electrophysiological Technique for the Diagnosis of TON in a Small Animal Model." Engineering in Health and Industry Research Symposium at The Ohio State University, 25 February 2023, Columbus, OH.
- Ryan, Annie K.; Landreth, Claire; Cheung, Rachael; Artis, Elizabeth; Rex, Tonia S.; Racine, Julie; Reilly, Matthew A. "An Electrophysiological Technique for the Diagnosis of TON in a Small Animal Model." Ophthalmic Engineering Grand Rounds, 1 June 2023, Columbus, OH.
- Ryan, Annie K.; Landreth, Claire; Cheung, Rachael; Artis, Elizabeth; Rex, Tonia S.; Racine, Julie; Reilly, Matthew A. "An Electrophysiological Technique for the Diagnosis of TON in a Small Animal Model." Engineering Brain Injury Solutions Symposium, 9 June 2023, Columbus OH.

Websites or Other Internet Site(s)
Nothing to report.

Technologies or Techniques
We have developed a novel RBI technique which will be featured in an upcoming scientific article. This technique directly enables investigation of RBI-administered candidate therapeutics for any ocular application.

Inventions, Patent Applications, and/or Licenses
Nothing to report.

Other Products
Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Individuals Contributing to the Project

Name:	Matthew Reilly
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	ORCID 0000-0001-8029-0084
Nearest person month worked:	3
Contribution to Project:	Dr. Reilly is the project PI and has overseen all aspects of the project.

Name:	Katelyn Swindle-Reilly
Project Role:	co-PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-1739-0263
Nearest person month worked:	1
Contribution to Project:	Dr. Swindle-Reilly has coordinated the hydrogel development, has attended meetings related to the research project, and has managed students performing experiments.

Name:	Julie Racine
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	0000-0003-4409-0936
Nearest person month worked:	1
Contribution to Project:	Dr. Racine has developed visual electrophysiology protocols, trained graduate students and employees to implement them, and analyzed electrophysiological data.

Name:	Annie Ryan
Project Role:	Graduate Research Assistant
Researcher Identifier (e.g. ORCID ID):	0000-0003-3645-8649
Nearest person month worked:	12
Contribution to Project:	Ms. Ryan has conducted electrophysiology experiments, injury modeling, immunohistochemistry, and data analysis.

Name: Stephanie Small
Project Role: Undergraduate Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2
Contribution to Project: Ms. Small has assisted with electrophysiology experiments, injury modeling, immunohistochemistry, and data analysis.

Name: Emma Lally
Project Role: Undergraduate Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2
Contribution to Project: Ms. Lally has assisted with electrophysiology experiments, injury modeling, immunohistochemistry, and data analysis.

Name: Eve Howard
Project Role: Undergraduate Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1
Contribution to Project: Ms. Howard has assisted with electrophysiology experiments, injury modeling, immunohistochemistry, and data analysis.

Name: Michelle Mosko
Project Role: Undergraduate Research Assistant
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 2
Contribution to Project: Ms. Mosko has assisted with electrophysiology experiments, injury modeling, immunohistochemistry, and data analysis.

Change in Active Other Support of PD/PI(s) or Senior/Key Personnel

Matthew Reilly, PD/PI is listed as senior/key personnel on the following new grants awarded since September 2022:

- NIH 2R01EY027399-06, Corneal Biomechanics in Ocular Disease: 2023-2027, $1,699,040. Role: Co-I
- NIH 1R01EY035278-01, Biomechanics of Lens Morphogenesis: 2023-2028, $1,972,794. Role: PI
- DOD Vision Research Program Investigator-Initiated Research Award HT94252310782, Sustained Therapeutic Protein Cocktail Delivery to Prevent Vision Loss After Ocular Trauma: 2023-2026, $1,158,179. Role: Co-PI
- NIH 1R01EB032870-01, Predictive Drug Release from a Tunable Injectable Capsule: 2023-2027, $2,062,484. Role: Collaborator

Katelyn Swindle-Reilly, co-PI is listed as senior/key personnel on the following new grants awarded since September 2022:

- NIH 1R01EB032870-01, Predictive Drug Release from a Tunable Injectable Capsule: 2023-2027, $2,062,484. Role: PI
- DOD Vision Research Program Investigator-Initiated Research Award HT94252310782, Sustained Therapeutic Protein Cocktail Delivery to Prevent Vision Loss After Ocular Trauma: 2023-2026, $1,158,179. Role: PI
- NIH U01EY032973, First aid medicine to treat vesicant-induced corneal injury: 2022-2027 $3,794,176. Role: PI
- Owen Locke Foundation, Tunable Extended-Release Capsule for Age-Related Macular Degeneration (TERC-AMD): 2022-2023 $150,000. Role: PI
- The Ohio State University UnEYEted Collaborative Funding, Evaluate polydopamine nanoparticle delivery system after intravitreal injection in a rodent model: 2023 $4,000. Role: co-PI
- NIH R21EY035035, The impact of age-related vitreous degeneration and vitreous replacement on scleral
- biomechanics: a novel mechanism and treatment target for glaucoma: 2023-2025. $425,145. Role: co-PI
- NIH R01EB032870, Predictive Drug Release from a Tunable Injectable Capsule: 2023-2027 $2,062,484. Role: PI
- NIH R01CA270169, Cerebrovascular-tumor-on-a-chip: Sustained adjunct therapy to improve chemotherapy efficacy in glioblastoma: 2023-2028 $3,078,719. Role: co-PI
- NIH R21EY035038-01, Evaluation of New Anti-inflammatory Treatments for Age-Related Macular Degeneration: 2023-2025 $433,124. Role: PI

Other Organizations Involved as Partners

Organization Name: Vanderbilt University
Location of Organization: Nashville, TN

Partner's Contribution to the Project: We have collaborated with Dr. Tonia Rex at Vanderbilt University. She has assisted with interpretation of immunohistochemistry data and will appear as a co-author on relevant manuscripts. She also trained Annie Ryan on the relevant techniques.

SPECIAL REPORTING REQUIREMENTS

Updated quad chart is attached.

APPENDICES

N/A