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TITLE: Development and Validation of a Novel Specialized Proresolving Mediators (SPM) Therapy to Counter Blast-Related Eye Injuries

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Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This proposed research project meets the intent of FY19 VRP IIRA and aligns with the Focus Areas: "Eye injury or visual dysfunction as related to a military-relevant traumatic event" due to blast exposure and "Diagnosis and treatment of eye injuries in austere environments and prolonged field care settings." The rationale for this proposal is based on the expected change in guidelines for combat eye care due to prolonged field care scenarios. Soldiers that have experienced closed globe injuries and visual system impairments after blast or to blast-related traumatic brain injury (TBI) are transferred to higher levels of care until the appropriate provider and capabilities are available. In potential future conflicts, delays in medical evacuation are expected, which will impact the ability to provide combat casualty care using current ocular trauma protocols. Visual dysfunction without severe ocular injuries are very common with blast-related TBI and can result from a myriad of causes to include direct damage to the retina, optic nerve and other neurosensory CNS structures of the brain. The objective of the proposed study is to examine the deficit on the visual system drive by blast injury, and the basis of the specialized pro-resolving lipid mediators (SPMs) as neuroprotective therapeutic interventions to mitigate tissue damage, neuroinflammation, and ameliorate visual deficit caused by blast exposure. Blast exposure causes retinal and optic nerve injury, which in turn leads to neuroinflammation as seen by increased inflammatory mediators, gliosis (microglial and astrocyte activation) and apoptosis.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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TABLE OF CONTENTS

1. INTRODUCTION:

Despite the growing clinical evidence on the broad subject of blast related traumatic brain injury (TBI) on the visual dysfunction and reports demonstrating blast-induced ocular injury, very few studies have evaluated therapeutic interventions on retinal and optic nerve damage due to blast exposure. Furthermore, few reports have investigated neuroprotective agents in blast mediated-retinal injury and -TBI models. Thus, the purpose of this study is to investigate an early therapeutic intervention that mitigates increased inflammatory and apoptotic mediators as well as glial activation as targets to suppress retinal and optic nerve injury due to blast exposure. In this research project we are investigating whether the specialized pro-resolving mediators (SPM) maresin 1 (MaR1) and neuroprotectin D1 (NPD1) can mitigate blast-induced retinal and optic nerve degeneration in a preclinical ocular trauma model. MaR1 and NPD1 have shown to promote inflammatory resolution, neuroprotection, and improve functional neurological recovery in several brain and spinal cord injury models. Moreover, our current data based on immunohistochemistry of the retinas and optic nerves of rats exposed side-ways to blast waves with peak overpressure of ~ 140 kPa demonstrated that MaR1 and NPD1 reduced glial fibrillary acidic protein (GFAP) and microglial-specific protein Iba-1 expression in the retina from blast-exposed rats compared to untreated subjects. We are currently conducting feasibility studies to assess deficits on the visual system driven by blast injury to correlate them with the efficacy of SPM agents in the retina, optic nerve, and optic tract after blast wave exposure. The proposed SPM treatment will result in improved histopathological and functional outcomes, such as diminishing the inflammatory response, reducing microglial neuroinflammatory processes, reducing retinal ganglion cell death, optic nerve degeneration, and preserving visual function.

2. KEYWORDS:

Neuroinflammation, specialized pro-resolving mediators, neuroprotection, blast-eye injury, traumatic brain injury, retina, optic nerve, maresin 1, neuroprotectin D1, gliosis, and microglial

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Milestone:

- 2020, Q1: Develop and submit animal protocol
- 2021, Q2: Optimization of blast eye injury model and functionality tests
- 2021, Q3: Conduct blast wave exposures to the rats and administer MaR1, and NPD1 post-blast exposure at different time points
- 2021, Q4: Currently testing electroretinograms (ERGs) and visual evoked potentials (VEPs) from experimental groups of rats including control, blast exposed, MaR1 and NPD1 treated after blast exposure

What was accomplished under these goals?

Accomplishments:

- An experimental model development study was conducted to determine the blast level to be used in treatment studies. Criteria were based on immunohistochemistry and Western blots analyses of the retinas and optic nerves of rats exposed to blast waves as compared to controls, figures 1-2.
- A time course study was conducted using the most effective dose of MaR1 and NPD1 given approximately 30 minutes after blast wave exposure of the rats.
- Assess histopathology of eyes and optic nerves from experimental groups of rats including control, blast exposed, and MaR1- and NPD1-treated after blast exposure
- Started ERG/VEP testing from experimental groups of rats including control, blast exposed, and MaR1- and NPD1-treated after blast exposure, figure 3.

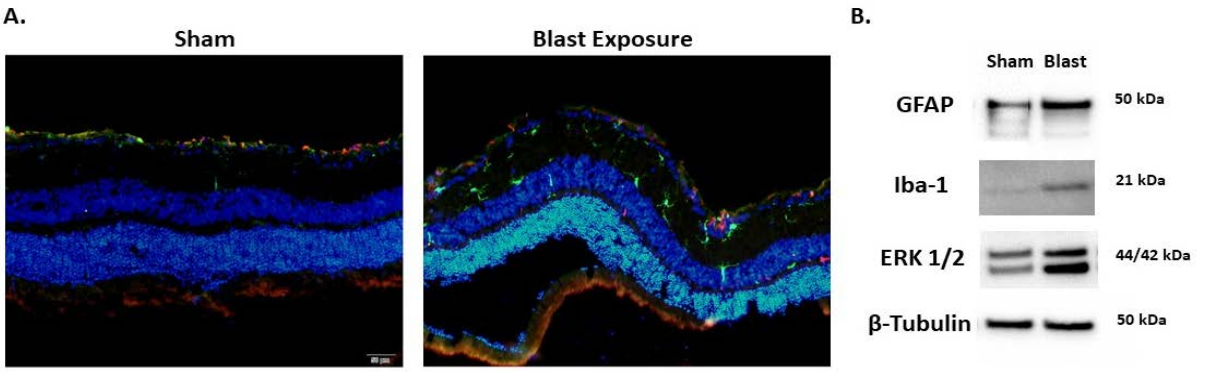

Figure 1: Immunostaining and Western Blots analyses of different inflammatory biomarkers in the retina after blast exposure. (A) Increased expression of GFAP and Iba-1 in the retina after blast exposure. **(B)** Increased protein levels of GFAP, Iba-1, and ERK 1/2 pathway activation, which induce an inflammatory response and exacerbates retinal injury after blast exposure. Magnification, 20X.

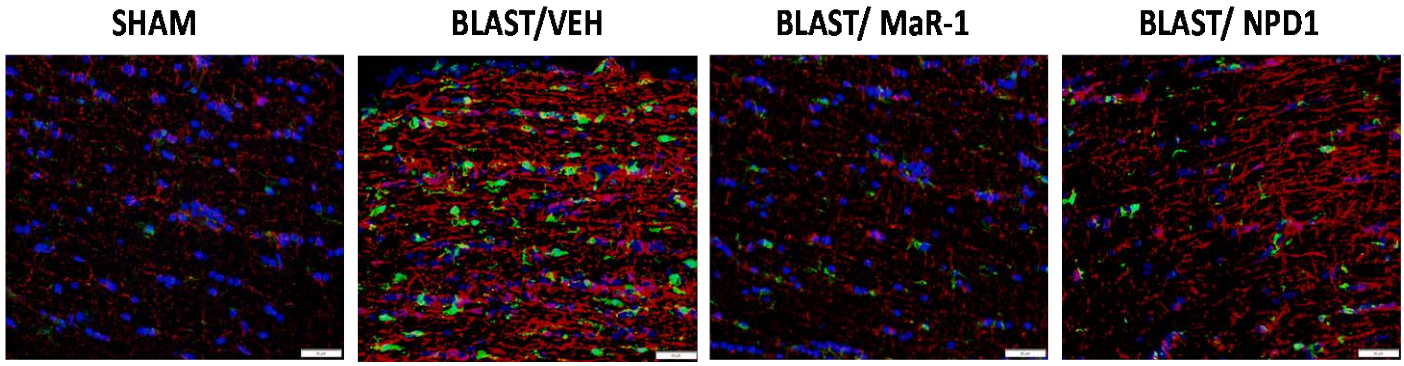

Figure 2: Immunolocalization of GFAP, Iba-1, and DAPI (nucleic marker) in the optic nerve (ON) after blast exposure and treated with either the vehicle (saline), MaR-1, and NPD1. Increased expression of GFAP and Iba-1 were observed in the ON after blast exposure and treated with saline as compared to MaR-1 and NPD1 treatments. Magnification, 20X.

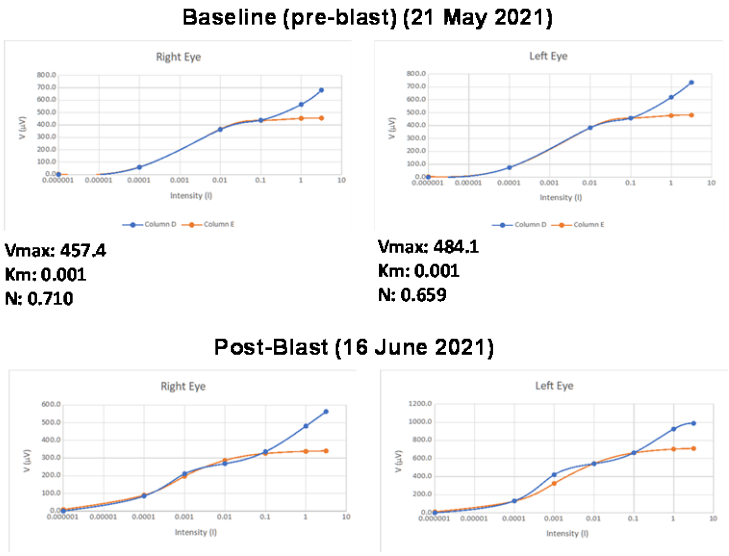

Figure 3, Progress in the electrophysiological assessments in a rat model of blast-induced ocular trauma. The figures represent data from animal ID 28082, the blue traces indicate the observed (measured) ERG response amplitudes, and the tan traces indicate the regression line fitted to the data with the Naka-Rushton function. The photopic ERG, as well as the flicker ERG, also indicated a loss of response amplitude in the blast-exposed eye. The VEP yielded variable results over the three post-blast exams in this animal, but indicated that the optic nerve maintained basic functionality at this blast level.

As noted in our original proposal and in the previous progress reports, we are using a combination of electroretinograms (ERGs) and visual evoked potentials (VEPs) in the rats to provide a quantitative measure of visual system impairment following the blast exposures. The rod and cone photoreceptor function are assessed by scotopic and photopic intensity-response flash stimuli, and the temporal response of the cone system is assessed by flicker ERG recordings over the range of 5 to 30 Hz (flashes/sec). The integrity of the optic tract (optic nerve, chiasm, geniculate nucleus relay synapses, and visual cortical responses) are assessed with a 2 Hz flash evoked VEP.

To assess the intensity-response scotopic ERG, we are using the Naka-Rushton function fit to the experimental data to obtain quantitative parameters of maximum rod response amplitude (Vmax, mV), stimulus intensity evoking a half-maximal response (Km, dB from the reference level), and a cooperativity factor (N, unitless). An example set of responses are illustrated in Figure 3. The changes observed in the Vmax and Km values in the blast-exposed eye, compared to the opposite eye, provide a clear, objective measure of the change in visual function.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

- Perform studies on caspase-3 labeling of retinal ganglion cells from experimental groups of rats including control, blast exposed, and MaR1- and NPD1-treated after blast exposure.
- Finalize ERG/VEP and OCT testing as well as the biochemical analyses (Western Blots, ELISA and multiplex).
- Submit two abstracts for poster presentations at The Association for Research in Vision and Ophthalmology (ARVO) 2022 and MHSRS 2022.
- Analyze data and prepare manuscript for peer review publications

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The results of the proposed study will advance knowledge for developing treatments for neurosensory trauma resulting in visual dysfunction in soldiers that suffer from ocular trauma and/or TBI due to blast injury. This research project may lead to novel treatment approaches for blast injury with NPD1 and /or MaR1 therapies. SPM can be used on the battlefield to reduce tissue damage from injury and inflammation by a novel mechanism stimulating endogenous resolution programs for reducing inflammation and pain without immune suppression as well as intensified treatment either at point of injury or until specialized care is available. Successful completion of this study will advance us closer to clinical translation for military-relevant ocular trauma and TBI. Thus, the experimental manipulation of SPM-mediated signaling to slow or halt the initiation and progression of neurodegenerative retinal and optic nerve ganglion cells represents an emerging target for pharmaceutical intervention and clinical translation.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

The current COVID-19 situation has impeded the performance and progress of this research project in our research institution, U.S. Army Institute of Surgical Research (USAISR). We have recently encountered a delay in our research project form more than one month as result of the unavailability of needed anesthetic supplies from the VSB facility at USAISR. The anesthetic at issue was out of stock and took 35 days for it became available despise our efforts to acquire sooner. The lack of anesthetic put on hold on ongoing experiment, and we must restart the experiment a month after. The anesthetic was approved by IACUC to be used in our animal protocol. We also experienced delays and difficulties in obtain a visitor pass for Dr. Glickman, who is a Co-PI in this project.

Changes in approach and reasons for change.
Actual or anticipated problems or delays and actions or plans to resolve them.

Dr. Ríos submitted an addendum on his animal protocol to IACUC requesting the addition of other anesthetics to prevent further issues with the lack of anesthetics from the VSB facilities at USAISR.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals
Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Nothing to Report.

  Books or other non-periodical, one-time publications.

  Nothing to Report.

  Other publications, conference papers and presentations.

  Nothing to Report.

- **Website(s) or other Internet site(s)**

  Nothing to Report.

- **Technologies or techniques**

  Nothing to Report.

- **Inventions, patent applications, and/or licenses**

  Nothing to Report.

- **Other Products**

  Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Jose Rios Garcia
Project Role:	PI
Researcher Identifier:	N/A
Nearest person month worked:	11.1
Contribution to Project:	Overseeing and carrying out the project protocol, collecting and analyzing data, and preparing and finalizing reports.
Name:	Randolph Glickman, PhD
Project Role:	Co-PI
Researcher Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Dr. Glickman has performed work with the setting of the retinal electrophysiological equipment to perform electroretinogram (ERG) on blast eye injury rats and analyzing ERG data.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

United States Army Institute for Surgical Research: site facilities.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS: Attached.

9. APPENDICES: None.