AWARD NUMBER: W81XWH-20-2-0026

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

PRINCIPAL INVESTIGATOR: José David Ríos

CONTRACTING ORGANIZATION: The Geneva Foundation, Seattle, WA

REPORT DATE: October 2022

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
October 2022	Annual	01Sep2021-31Aug2022

4. TITLE AND SUBTITLE

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

5a. CONTRACT NUMBER
-

5b. GRANT NUMBER
W81XWH-20-2-0026

5c. PROGRAM ELEMENT NUMBER
-

6. AUTHOR(S)
José David Ríos
Jose.d.rios10.ctr@mail.mil

5d. PROJECT NUMBER
-

5e. TASK NUMBER
-

5f. WORK UNIT NUMBER
-

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Geneva Foundation
917 Pacific Avenue Suite 600, Tacoma, WA 98402
3698 Chambers Pass Bldg. 3611, JBSA Fort Sam Houston, TX 78234

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
USAMRAA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
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**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	14	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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?

Major Tasks:

1. Conduct initial feasibility studies in order to assess deficit on the visual system driven by blast injury.

2. Evaluate the efficacy of neuroprotective SPM agents in the retina, optic nerve, and optic tract after blast wave exposure.

3. Determine the effects of SPMs intervention on blast-induced tissue damage in the retina and optic nerve.

What was accomplished under these goals?

Accomplishments:

- Subtask 1.1: Submit animal protocol documents for institutional IACUC review. **(100% Complete)**
  - Developed and submitted animal protocol; Submitted 22 SEP 2020
- *Milestone #1: IACUC approval received and completion of the feasibility studies* **(100% Complete)**
  - IACUC Approved 02 DEC 2020, ACURO Approved 17 DEC 2020
- Subtask 1.2: Optimize the blast intensity needed to achieve the desired retinal and optic nerve injury severity in terms of visual function and associated morphological changes by using fERG/fVEP and OCT analysis. **(100% Complete)**
- *Milestone #2: Complete the optimization of blast eye injury model.* **(100% Complete)**
  - 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.
- Subtask 2.1: Validate SPMs purity and HPLC signature from commercial sources by LC-MS/MS. **(100% Complete)**
- Subtask 2.2: Examine the effects of SPMs on changes in visual function and retinal thickness after blast exposure using fERG/fVEP and OCT analyses. **(fERG data analysis for retina 100% Complete, fVEP data analysis for optic nerve 20% Complete)**
  - Assessment of ocular function using scotopic electroretinogram (ERG) indicated loss of retinal response amplitude after blast, which was mitigated by MaR1 and NPD1 intervention.
- Subtask 2.3: Determine SPMs levels in retina and optic nerve samples after blast injury by LC-MS/MS. **(Task Not Performed)**
- Subtask 2.4: Examine the effects of SPMs on increased neuroinflammatory-related biomarkers in the retina and optic never tissues after blast injury using multiplex, ELISA, and immunohistochemistry analyses. **(Retina 100% Complete, Optic Nerve 80% Complete)**
  - Blast exposure resulting in retinal damage, manifested by increased expression of proteins involved in gliosis, was reduced by MaR1 and NPD1 intervention.
- Subtask 2.5: Examine the effects of SPMs on microglia modulation in the retina, optic nerve, and optic tract after blast exposure by immunohistochemistry analysis **(100% Complete)**
  - Assessed histopathology of eyes, optic nerves, and brain's optic chiasm regions from experimental groups of rats including control, Vehicle-blast exposed, and MaR1- and NPD1-treated after blast exposure.
    - Based on immunohistochemistry of the retinas and optic nerves of rats exposed side-ways to blast waves with a 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 and optic never from blast-exposed rats compared to untreated subjects.
- *Milestone #3: Co-author manuscript on the pathophysiologic mechanism of blast-related ocular injury and the efficacy of proposed SPM treatments* **(In Preperation)**
- Subtask 3.1: Examine the effects of SPMs on blast exposure induced increased of caspase-3 activation in the retina optic never and optic by immunohistochemistry. **(Caspase-3 activation analysis for retina 100% Complete, Caspase-3 activation analysis for optic nerve 20% Complete)**
- Subtask 3.2: Examine the effects of SPMs on blast exposure induced increased of apoptosis in the retina optic never and optic using TUNEL assay. **(Task Not Performed)**
- *Milestone #4: Co-author manuscript on the effect of SPM on the blast-induced ocular trauma with apoptosis in the retina and optic nerve* **(In Progress)**

In this research project, a blast-related eye injury model by using a compressed air-driven shock tube allowed us to investigate whether retinal, optic nerve, and brain tissues exhibited pathological changes for acute post-blast efficacy study and identify best treatment dose for long-term post-blast study. This model was used for subsequent neuroprotection studies to counter the effects of blast overpressure on the retina and optic nerve, an area of great interest for military medicine. Our injury model demonstrated that exposures to a blast wave peak overpressure of 140 ± 5 kPa with a positive-phase overpressure impulse (impulse per unit surface area) of 143.0 ± 9.2 kPa-ms and a positive pressure phase duration of 3.12 ± 0.03 ms induced an increase in retinal thickness along with increasing in astrocytes and microglia activation, and a significant increase in local pro-inflammatory cytokines in the rat retina after blast exposure. These pathological changes are consistent with previous reports where increases in retinal thickness, glial fibrillary acidic protein (GFAP) expression, glutamate levels, and caspase-3 activation corresponded with increases in local inflammatory cytokines and chemokines in the rat retina as early as 24 h after blast exposure.

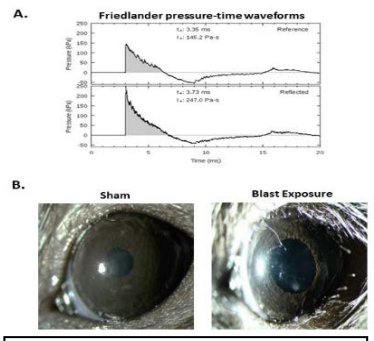

Figure 1. Feasibility of a blast-related eye injury model and the proposed evaluation of therapeutics. A) Graph of typical overpressure wave profile from USAISR air–driven shock tube; B) slit-lamp examination right after blast exposure.

Under slit-lamp examination, corneal abrasions and transient corneal and lens opacities were observed immediately after blast wave exposure in the left eye that was facing the blast wave, which resolved at 48 h post-blast. Moreover, a persistent asymmetric pupil's contraction in response to the light was observed in most of the blast exposed rats (**Figure 1B**). This abnormality may relate to either structural or neurological damage to the iris sphincter muscles and their innervation. There were also animals that presented hyphemia and retinal hemorrhage immediately after blast exposure that were associated with a tertiary blunt effect from blast exposure. No other visible injuries or conditions affecting corneal transparency were observed during baseline or at 48 h after blast wave exposure. The retinal thickness (RT) measurements obtained via optical coherence tomography (OCT) 48 h post-blast was greater in blast exposed animals as compared to control animals (**Figure 2A & B**). Of note, we observed an increase in retinal thickness in the animals exposed to blast compared to their sham or control counterparts. We further investigated whether edema mediates increasing in retinal thickness after blast injury, looking at the expression of aquaporin 4 (AQP4), a membrane water channel protein. Changes in the expression of AQP4 have been linked to edema in blast-related retinal injury. Increased expression of AQP4 was observed in retinal tissue by immunohistochemical analysis and in retinal homogenates of blast exposed rats as compared to sham, suggesting an acute retinal edema process in response to blast injury (**Figure 2C&D**). We further investigated whether MaR1 and NPD1 Reduce Glial Cells Activation in the Retina and

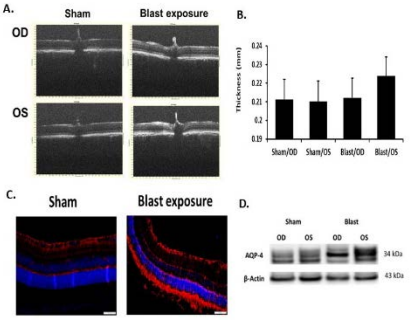

Figure 2. Increased retinal thickness and increased expression of aquaporin 4 (AQP4) proteins involved in edema at 48h after blast exposure. A) Representative OCT imaging retinal thickness between blast exposed rats and their counter control B) Measurement of total retina thickness. C) Immunolocalization of AQP4. D) Western blot analysis of AQP4 at 48h after blast exposure. Data are presented as the

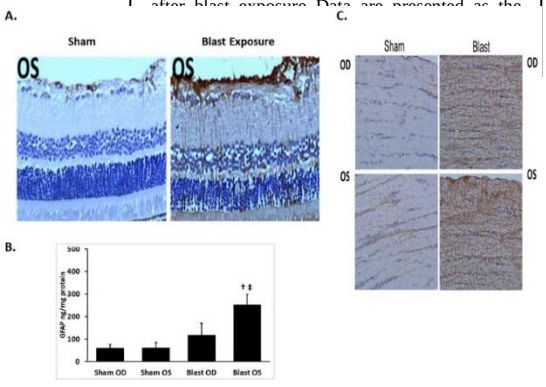

Figure 3. Increased expression of GFAP in the retinal and optic nerve (ON) tissues at 48h after blast exposure. A) Immunohistochemical localization of GFAP on the retina, left eye (OS). B) Measurement of total GFAP protein expression by ELISA. C) Immunohistochemical localization of GFAP in the ON after blast exposure. Data are presented as the mean of 3 experiments. Data are presented as the mean of 3 experiments. * indicates $p < 0.05$.

Optic nerve after Blast Exposure. Activation of Müller glia, indicated by an increase in the distribution of GFAP immunoreactivity, is the ubiquitous response to stress observed in various retinal degenerative diseases and retinal injury after exposure to blast overpressure. GFAP protein levels were significantly higher in blast exposed rats compared to sham rats ($P < 0.05$; **Figure 3B**). GFAP upregulation was observed at 48 h, acutely post-blast (**Figure 3**) as well as long-term post-blast at 9, 23, and 37 days, (**Figure 5**). We observed a continuous increase in GFAP immunolabeling in the nerve fiber layer and ganglion cell layer (NFL/GCL) toward the outer retina layer and in the optic nerve of the left eye after blast exposure as compared to the sham. Interestingly, the contralateral eyes that were not directly exposed to the blast wave also presented increase in GFAP expression in the optic nerve at 48 h after blast (**Figure 3C**).

The upregulation of GFAP in Müller cell processes in the outer retina layer of the left eye suggests blast-related damage to the retina. Similar effects on Müller glial cell response have been reported by Zou et al in primary blast-model that yielded NFL/GCL injury. We investigated the efficacy of MaR1 and NPD1 post-blast in the short-term. Using a dose-response study for MaR1 or by using a single dose of NPD1 administered ~30 min after injury, we found that acute MaR1 treatment significantly reduced GFAP protein levels in a dose-dependent manner from blast-exposed animals at 48 h post-blast (**Figure 4**). Notably, 1 µg/kg MaR1 seemed the most effective dose, which was used in subsequent long-term neuroprotective studies. Moreover, 0.1 µg/kg NDP1 reduced GFAP levels from retinal homogenates of rats exposed to blast compared to sham rats. These observations were in agreement with a recent study which reported that MaR1 inhibited the activation of microglia and astrocytes in a dose range of 0.01 to 1 µg/kg/day when administered within 1 h post-injury in models of spinal injury and neuropathic pain.

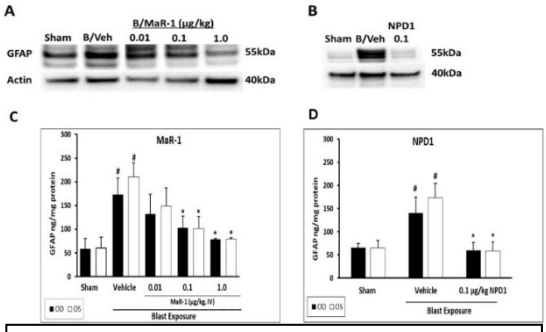

Figure 4. Efficacy of Maresin 1 (MaR1) and Neuroprotectin D 1 (NPD1) in retina at 48h after blast exposure: Western Blots and ELISA. A-B) Representative Western blots as dose-response for MaR-1 and at 0.1ug/kg of NPD1. C-D) Measurement of total amount of GFAP expression by ELISA. Data are presented as the mean of 3 experiments. * indicates $p < 0.05$.

We further investigated long-term or chronic injury mechanisms manifested by retinal and optic nerve glial cell activation, inflammation, and vision dysfunction after the administration of MaR1, NPD1, or vehicle, with a follow-up at 9, 23, and 37 days after blast injury. To determine the impact of MaR1 and NPD1, we analyzed glial cell activation in the retina and optic nerve as the hallmark of reactive gliosis and neuroinflammation by the immunolocalization of GFAP for Müller Cells, "macroglia" and ionized calcium binding adaptor molecule 1 (Iba-1) for microglia/macrophages, respectively. Low levels of GFAP-reactive and Iba-1 positive cells are visible in the retina from sham/control animals. An increase in GFAP immunoreactivity was observed in the NFL /GCL of retinas from blast-vehicle animals as compared to control animals at 9-, 23-, and 37-days post-blast. Furthermore, increased Iba-1 positive microglia were detectable throughout all layers of retina from blast-vehicle animals at 9-, 23-, and 37-

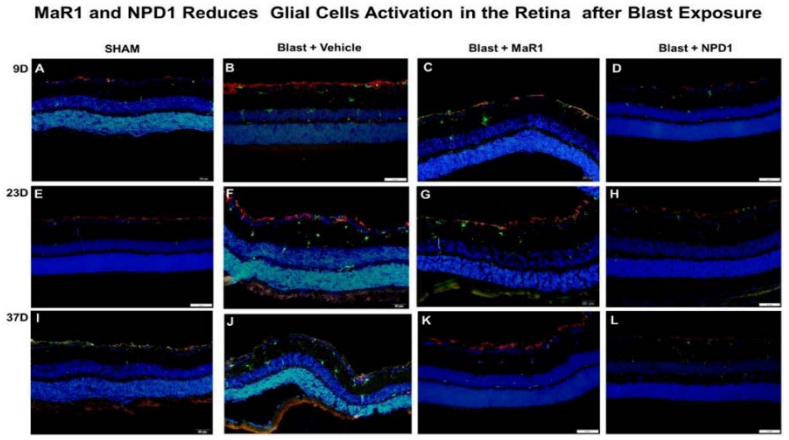

Fig 5. Immunolocalization of GFAP and Iba-1 in the retina after blast exposure. Increased GFAP and Iba1 immunostaining in the retina at 9-, 23-, and 37-days post-blast. The extent of GFAP and Iba-1 immunostaining increased across the retina of vehicle + blast-exposed rats (B-C) compared to control animals (A-I). GFAP and Iba-1 positive cells decreases in either MaR1 and NPD1 treated animals after blast exposure (C-L) GFAP immunostaining positive cells (red), Iba1 immunostaining positive cells (green) and DAPI for nuclei staining (blue); 100X magnification (bards = 100 µm).

days post-blast. However, there was a decrease in the number of GFAP and Iba-1 positive cells observed in retinas from animals treated with 1.0 μg/kg/day MaR1 or 0.1 μg/kg/day NPD1 after blast exposure **(Figure 5)**.

These results from work in progress indicate that MaR1 and NDP1 ameliorate reactivity for GFAP and Iba-1 in retinal cell layers after blast exposure. Increased expression of these proteins involved in gliosis after blast that was significantly reduced with treatment was confirmed by Western blot analysis. MaR1 and NPD1 treatment significantly reduced GFAP expression in retinal homogenates at day 9, 23, and 37 post-blast injury, suggesting neuroprotective effects of treatment after blast exposure (**Figure 6**). Increased GFAP immunoreactivity was also detected in the optic nerve after blast exposure, which was ameliorated by MaR1 and NPD1 (**Figure 7**). Moreover, microglia activation shown by increased expression of the microglial-specific protein, Iba-1, in the retina suggest that microglia are responding to blast-induced retinal ganglion cells (RGC) damage and that their activation is contributing further to neurodegeneration and cell death. Microglia can be activated by RGC damage, resulting in proliferation and migration to areas of damage within the retina and the optic nerve. The morphological activation and increased number of Iba-1 positive cells observed in our model suggest that microglia are responding to blast-induced RGC damage and that their activation is contributing further to neurodegeneration and cell death. Microglia comprise the majority of tissue macrophage population within the central nervous system, therefore, its activation represents a common pathway of mechanism in a variety of retinal degenerative diseases, often parallel to chronic inflammation and the onset of retinal cell death. After mild TBI, M1-activated microglia often release inflammatory cytokines and other toxic substances, worsening outcomes. Microglia have two distinct polarization states; pro- or anti-inflammatory states. The microglial pro-

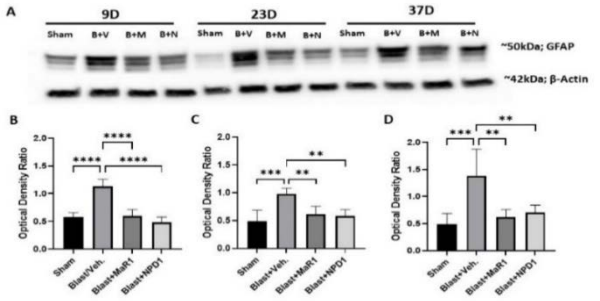

Fig 6. MaR1 and NPD1 Reduces GFAP and Ib-1 Protein Levels in the Retina after Blast Exposure A) Western blot analysis from rat's retinal protein lysates at day 9, 23, and 37 after blast exposure. B-D) Densitometry scans of protein levels of Western blots. GFAP levels were increased in retinal lysates from blast exposed rats. MaR1 and NPD1 significantly decreased GFAP protein levels at day 9, 23, and 37 after blast exposure. Data are expressed as means – standard error of the mean. n = 5, *p < 0.05.

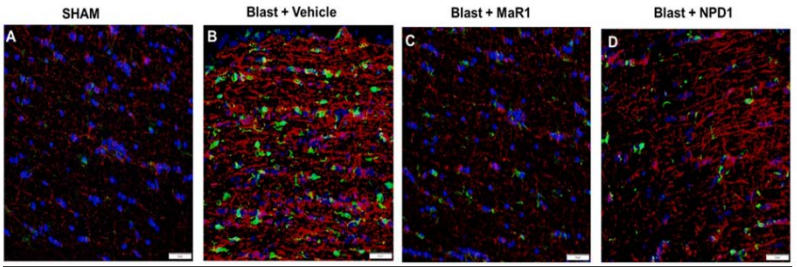

Fig 7. Immunolocalization of GFAP and Iba-1 in the optic nerve (ON) at day 9 after blast exposure. MaR1 and NPD1 reduces glial cells activation in ON tissue after blast exposure. Increased GFAP and Iba1 immunostaining in the ON of blast + vehicle animals (B) compared to control (sham) animals (A). The extent of GFAP and Iba-1 immunostaining decreased across the ON at day 9 after blast exposure in blast+Mar1 and blast + NPD1 treated animals (C-D). GFAP immunostaining positive cells (red), Iba1 immunostaining positive cells (green) and DAPI for nuclei staining (blue); 200X magnification (bards = 50 μm).

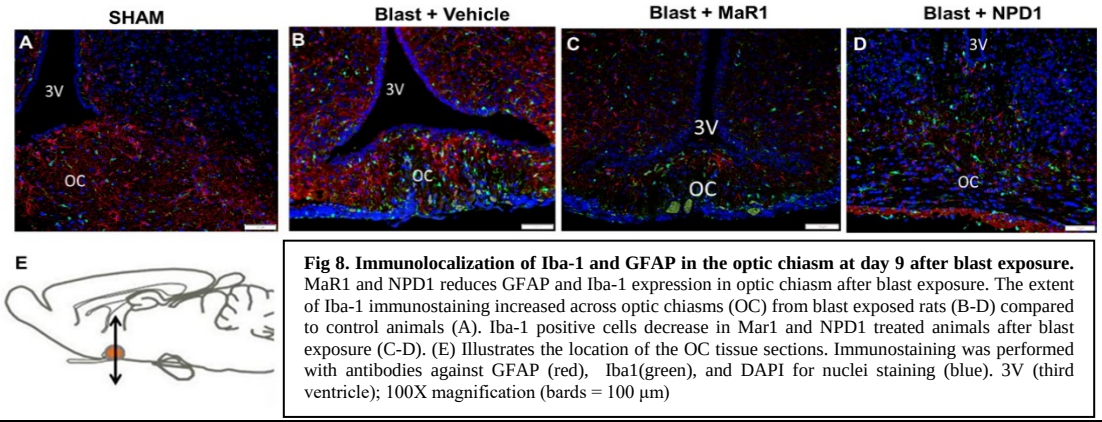

Fig 8. Immunolocalization of Iba-1 and GFAP in the optic chiasm at day 9 after blast exposure. MaR1 and NPD1 reduces GFAP and Iba-1 expression in optic chiasm after blast exposure. The extent of Iba-1 immunostaining increased across optic chiasms (OC) from blast exposed rats (B-D) compared to control animals (A). Iba-1 positive cells decrease in Mar1 and NPD1 treated animals after blast exposure (C-D). (E) Illustrates the location of the OC tissue sections. Immunostaining was performed with antibodies against GFAP (red), Iba1(green), and DAPI for nuclei staining (blue). 3V (third ventricle); 100X magnification (bards = 100 μm)

inflammatory M1 state is a reasonable therapeutic target to modulate and convert to the M2 anti-inflammatory state. In the eye, microglia also tend to increase in numbers and migrate through the inner retina toward the site of injury using the Müller glia as scaffolding, in the case of photoreceptor damage, or toward the optic nerve head and nerve fiber layer, in the case of optic nerve damage. Microglial activation maybe a potential target for migrating damage to the eye and optic nerve after blast. Furthermore, evidence of astrogliosis as shown by increased GFAP expression and microglia activation in the optic nerve, optic nerve projections at the suprachiasmatic level with blast exposure (**Figure 8**) suggest neuroinflammation in subcortical targets of RGC axons. MaR1 and NPD1 administration each attenuated these changes, thus early administration of MaR1 and NPD1 can be an effective strategy to reduce retinal and optic nerve injury associated with gliosis due to blast wave exposures.

We further elucidated whether MaR1 and NPD1 contribute to retinal cell survival after blast-induced ocular trauma looking at evidence of apoptosis through the detection of activated caspase 3 in ocular tissues. Caspases are cysteine aspartate proteases that can be divided into two major phylogenic subfamilies, either interleukin (IL)-1β-converting enzyme (inflammatory) or mammalian counterparts of CED-3 (apoptotic) caspases. In this study, retinal tissues from rats exposed to blast overpressure were processed for immunohistochemistry and colorimetry assay to detect activated caspase 3, which is the final executioner of the apoptotic sequence. The antibody used in this study only recognizes activated caspase 3, enabling identification of apoptotic cells. Tissues were then evaluated for relative levels of positive signal as compared to sham or control. As shown in **Figure 9A**, retinal tissues from the rat's left side exposed to blast overpressure had increased levels of activated caspase 3 as indicated by the brown staining. At 48 hours, the retinal inner nuclear layer from the left side had a significant increase of activated caspase 3 cells, **Figure 9B**. In a long-term study, an increase in caspase-3 level was detected at 9-, 23-, and 37-days after blast exposure. MaR-1 significantly reduced capase-3 levels at 9- and 37-days after blast exposure, **Figure 9C**. NPD1 treatment seen to reduced caspase-3 level in retinal homogenates at day 9, 23, and 37 post-blast injury. The results of this study suggest a potential neuroprotective effect of the SPMs as caspase-targeting interventions on blast-induced apoptosis in retinal tissues. However, further works by using Tunnel assay are necessary to confirm our results. Ongoing work is also assessing blast effects on axonal degeneration of optic nerve fibers death due to apoptosis.

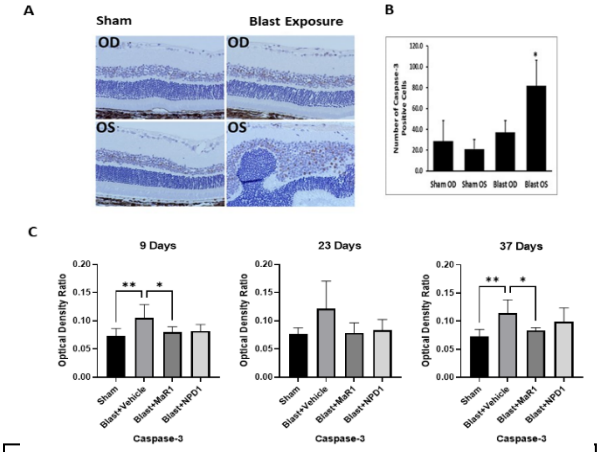

Fig 9. Caspase 3 activation in retina following blast exposure. (A) Increased expression of activated caspase 3 (brown staining) at 48 hours after blast exposure. (B) Quantification of caspase 3+ cells per field of view. (C) Quantification of caspase-3 levels at 9-, 23-, and 37-days post blast exposure. There was a significant increase of caspase 3+ cells (P = 0.05) at 48 h after blast exposure as compared to sham or control. There was a significant increase of caspase level from retinal homogenates (P = 0.05) at 9- and 37-days after blast exposure as compared to control. MaR-1 significantly (P = 0.05) reduced caspase-3 levels at 9- and 37-days after blast exposure.

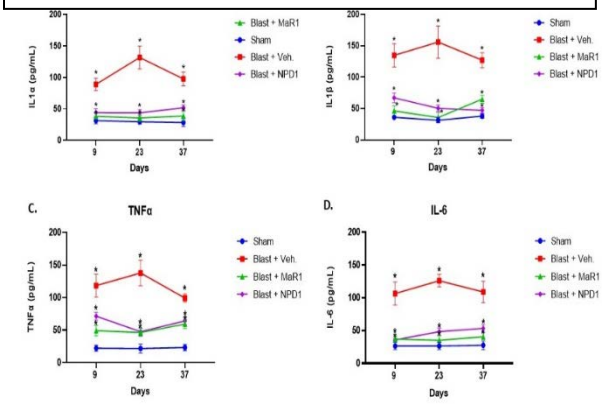

Fig 10. Retinal expression of inflammatory markers increased at 9-, 23, and 37-days post-blast was decreased by MaR1 and NPD1. (A-D) Increased expression of interleukin (IL)-1b, IL-1a, tumor necrosis factor (TNF)a, and IL-6 in the ipsilateral retinas of blast-injured rats was seen when compared to sham, MaR1, and NPD1 values at 9-, 23, and 37-days injury. Data expressed as means – standard error of the mean. n = 3 and four rats per group at 9-, 23, and 37-days post-blast, respectively. *p < 0.05.

We then investigated whether MaR1 and NPD1

treatment reduce proinflammatory cytokine levels after blast exposure. An increase in cytokines in the retinal tissue has been observed as a robust blast intensity-dependent inflammatory response after blast exposure. Local cytokine expression in retinal tissue was measured by multiplex analysis at 9-, 23- and 37-days post-blast. Increased retinal expression of interleukin (lL)-1β, IL-1α, IL-6, and tumor necrosis factor-α (TNFα), which was associated with activation of microglia and macroglia reactivity, respectively was observed in blast-vehicle rats when compared with sham, (**Figure 10**). Decreased cytokine expression and glial activation was accomplished after MaR1 or NPD1 administered IV ~30 min after blast injury and continued daily for one-week post-injury. The results revealed that treatment with MaR1 and/or NPD1 significantly reduced pro-inflammatory cytokines, implying that these SPMs may attenuate the inflammatory response due to blast exposure. Of note, levels of pro-inflammatory cytokines in the retina from blast-vehicle rats were sustained up to 37 days post blast suggesting an ***impairment in resolution of inflammation within the retina*** after blast exposure. Our data from work-in progress demonstrates that treatment with either MaR1 and/or NPD1 significantly reduced cytokines and gliosis, implying that these SPMs may attenuate inflammatory response due to blast exposure.

The modulated production of pro-inflammatory cytokines might partially explain the SPMs-induced decrease in the recruitment of activated microglia/macrophages as primary executors in the process of retinal inflammation associated with blast injury. An impairment in the inflammatory resolution to exacerbate an immunological cascade as well as reactive microglia may significantly contribute to furthering retinal and optic nerve tissue damage. Consistent with these observations, our preliminary immunohistochemical analyses shows activation of Iba1 positive microglia localized on the outer and inner plexiform layers of retinas exposed to blast. It is also tempting to speculate that MaR1 or NPD1 may modulate microglia phenotype from a detrimental phenotype (M1) to a beneficial phenotype (M2) that suppresses neuroinflammation and promote retinal repair as well as promoting the inflammatory resolution and wound healing of the retina after blast injury.

We further investigated injury mechanisms manifested by vision dysfunction after blast exposure and the administration of MaR1, NPD1, or vehicle. Blasted-treated and sham rats were given an electroretinogram (ERG) exam prior to blast wave exposure to establish their baseline light stimulus responses, and then retested once at 9-, 23-, and 37-days post blast. Rats were adapted in full darkness overnight, prior to being ERG tested. The eyes were then given a scotopic full field flash ERG exam, using a light stimulus procedure that exposes the eyes to a series of white light flashes of six increasing intensities (0.1, 0.3, 1, 3, 10, and 25 cd.s/m2), with each repeated one to four times (and the results averaged) at an interval of 10 s and a duration of 5 ms, and having a ramp spacing of 30–60 s. Peak amplitudes for a- and b-wave responses only at the light flash intensity of 3 cd s/m2 were used to evaluate overall ERG data with time. Analysis of scotopic ERG was performed by using the Naka-Rushton equation**.** The form of the Naka-Rushton equation is given as: **V=Vmax In/(In+Kn),** where **V** is the b-wave amplitude elicited by stimulus intensity; **Vmax** is the asymptotic value of the b-wave amplitude; **I** is the stimulus luminance; **K** is the stimulus luminance eliciting a half-maxim a l b-wave; and **n** is a dimension less fitting constant affecting the slope of the linear portion of the V-logI curve of the b-wave response. The equation was fit to the ERG data using the"Solver"non-linear regression add-in function available in both MSExcel and LibreOffice Calc; both programs worked equivalently. The Vmax values in the SPM treated eyes were the same or greater after blast exposure as at baseline, whereas Vmax declined in blast-untreated eyes at 23- and 37-days post blast (**Figure 11**). The K values after SPM treatment did not

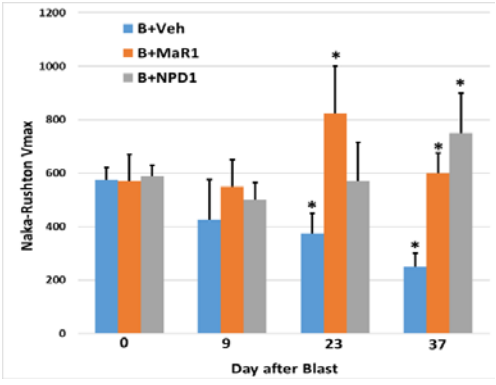

Fig 11. Treatment Effect on Scotopic Electroretinogram (ERG) Response after Blast Exposure. Effect on the Vmax parameter by mean of Naka-Rushton analysis. The Vmax values in the SPMs treated eyes were the same or greater after blast exposure as at baseline, whereas Vmax declined in untreated eyes (blast + vehicle). Asterisk: p~0.01.

differ significantly from the baseline after blast exposure. Thus, assessment of ocular function using scotopic ERG indicated loss of retinal response amplitude after blast, measured as a decrease in the Vmax. In animals treated for 1 week with MaR1 or NPD1, the loss of Vmax was mitigated (**Figure 10**), while the K parameter, as a surrogate of sensitivity, was not significantly affected by blast or SPM treatment. Our data suggest that an injury mechanism manifested by rod dysfunction after blast exposure compromise visual function, which was mitigated by MaR1 and NPD1. Ongoing work is assessing blast effects on cone function using the photopic ERG and optic nerve integrity using the VEP.

Overall, these studies demonstrate the value and feasibility of characterizing the SPM in blast-related ocular injury. Our experimental shock-tube blast injury model recapitulates the deregulated structural and physiological conditions developed in humans exposed to an Improvised Explosive Devices (IED). Our findings demonstrate that retinal and optic nerve damage, manifested by activation of microglia and astrocyte and increased expression of pro-inflammatory cytokines caused by blast forces were reduced by MaR1 and NPD1.Thus, the treatments of MaR1 or NPD1 suggest an effective strategy to reduce or halt retina and optic nerve glia cells activation due to blast-related eye injury. These findings may lead to potential targets for pharmaceutical intervention and clinical translation to protect the vision of Soldiers exposed to explosive devices in the combat field or during training, such as breacher training exposes Soldiers to multiple blast exposures, potentially leading to worse structural and functional visual outcomes. Thus, further investigation using this model is necessary to provide new insight into the potential of SPMs-mediated neuroprotection in acute and/or chronic events after blast exposure. It would also allow us to further analyze the potential impact of selected SPMs, on RGC loss and changes to visual acuity secondary to the blast trauma.

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

Nothing to Report.

How were the results disseminated to communities of interest?

Two posters were presented at the Association for Research in Vision and Ophthalmology (ARVO) annual meeting, held in Denver, Colorado, from May 1-4, 2022.

- Ríos JD, Edsall P, Golden D, Butler J, Szczesniak A, Pearson TJ, Crespo-Cruz H, Serhan CN, Glickman RD Specialized Pro-Resolving Lipid Mediators, Maresin 1 (MaR1) and Neuroprotectin D1 (NPD1) Reduce Gliosis in Retina and Optic Nerve after Blast Exposure. ARVO, vol. 63, 707-Fo232, June 2022
- Glickman RD, Golden D, Edsall P, Butler J, Szczesniak A, Pearson TJ, Crespo-Cruz H, Serhan CN, Ríos JD. Specialized Pro-Resolving Lipid Mediators, Maresin 1 (MaR1) and Neuroprotectin D1 (NPD1), Preserve Retinal Rod Function After Ocular Blast Trauma. ARVO, vol. 63, 698-Fo223, June 2022

A Manuscript, entitle: *"Specialized Proresolving Mediators (SPM) Ameliorate Neuroinflammation of Retinal Injuries Resulting from Blast Exposure"* is in preparation for submission in FASEB.

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

- To further extend the findings of this research project, the PI has submitted a VRP Level II proposal to the CDMRP. **Our goal is to advance novel and innovative therapies with interventions that target the resolution of inflammation to manage and treat ocular injury and visual dysfunction due to blast exposure swiftly in the battlefield.**

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 COVID-19 situation delayed the progress of our research project. Furthermore, the operational restructuration in our research institution, U.S. Army Institute of Surgical Research (USAISR) affected the timely manner of progress on deliverables. Thus, the Geneva Foundation requested a 12-month no cost extension on the subject referenced award. A 4-month NCE dated 30 NOV 2022 was approved through 31 DEC 2022.

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

An Addendum on adding the anesthetic adjuvant Xylazine as alternative to the protocol A-21-003 was requested on September 09, 2021 and approved by IACUC on September 13,2021.
This change was 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

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

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

Journal publications.

A Manuscript, entitle: *"Specialized Proresolving Mediators (SPM) Ameliorate Neuroinflammation of Retinal Injuries Resulting from Blast Exposure"* is in preparation for submission in FASEB.

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

Nothing to Report.

Other publications, conference papers and presentations.

Two posters were presented at the Association for Research in Vision and Ophthalmology (ARVO) annual meeting, held in Denver, Colorado, from May 1-4.

- Ríos JD, Edsall P, Golden D, Butler J, Szczesniak A, Pearson TJ, Crespo-Cruz H, Serhan CN, Glickman RD Specialized Pro-Resolving Lipid Mediators, Maresin 1 (MaR1) and Neuroprotectin D1 (NPD1) Reduce Gliosis in Retina and Optic Nerve after Blast Exposure. ARVO, vol. 63, 707-Fo232, June 2022
- Glickman RD, Golden D, Edsall P, Butler J, Szczesniak A, Pearson TJ, Crespo-Cruz H, Serhan CN, Ríos JD. Specialized Pro-Resolving Lipid Mediators, Maresin 1 (MaR1) and Neuroprotectin D1 (NPD1), Preserve Retinal Rod Function After Ocular Blast Trauma. ARVO, vol. 63, 698-Fo223, June 2022

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:	8.1
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.