AWARD NUMBER: W81XWH-22-1-0839

TITLE: Concomitant Topical Treatment of Pain and Inflammation in Ocular Injuries

PRINCIPAL INVESTIGATOR: Dr. Reza Dana

CONTRACTING ORGANIZATION: Schepens Eye Research Institute, Boston, MA

REPORT DATE: October 2023

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 2023	Annual	01Sep2022-31Aug2023

4. TITLE AND SUBTITLE

Concomitant Topical Treatment of Pain and Inflammation in Ocular Injuries

5a. CONTRACT NUMBER
W81XWH-22-1-0839

5b. GRANT NUMBER
VR210139

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Reza Dana

E-Mail: reza_dana@meei.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

SCHEPENS EYE RESEARCH INSTITUTE INC
20 STANIFORD ST
BOSTON MA 02114-2508

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)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

Our findings were summarized in two abstracts presented at ARVO 2023 titled: 1) NK1R Inhibition: a Novel Ocular Pain Management Strategy Following Mechanical Corneal Injury, 2) Effect of Neurokinin 1 Receptor Antagonist on Pain Response in Corneal Alkali Burn.

14. ABSTRACT

Corneal injuries being exceptionally painful and debilitating, often trigger a severe inflammatory response, leading to permanent visual damage. Substance P (SP) - a neuropeptide is known for its implication in inflammation and nociception. We hypothesize that the antagonism of neurokinin -1 receptor (NK1R), SP's preferred receptor, can suppress nociception, reduce inflammation, and promote wound healing following corneal injuries. We aim to study NK1R antagonist topical treatment in two injury models: mechanical and chemical (alkali burn). In aim 1, we focus on the analgesic efficacy of NK1R antagonism and its effect on corneal nerves. In aim 2, we explore the anti-inflammatory properties of NK1R antagonism and its effect on corneal wound healing. Our findings have revealed that elevation of SP in the cornea is correlated with pain sensation in corneal injuries. Inhibiting SP receptor - NK1R helps treat ocular pain following mechanical and chemical corneal injuries.

15. SUBJECT TERMS
Ocular Pain, Substance P (SP), Neurokinin-1 Receptor (NK1R) Antagonist, Mechanical Injury, Alkali Burn, Nociception, Eye-Wiping Test (EWT), Palpebral Ratio (PR) Nerve Regeneration, Neuroinflammation, Wound Healing

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	15	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

	Page
1. Introduction	1
2. Keywords	1
3. Accomplishments	1
4. Impact	5
5. Changes/Problems	7
6. Products	8
7. Participants & Other Collaborating Organizations	10
8. Special Reporting Requirements	12
9. Appendices	12

1. INTRODUCTION:

Our research seeks to establish a novel treatment for ocular pain following ocular injuries. Particularly, corneal injuries often trigger a severe inflammatory response, leading to permanent visual damage. Corneal injuries are exceptionally painful and debilitating due to the cornea's dense sensory innervation. Substance P (SP) is a well-known neuropeptide implicated in inflammation and nociception. We hypothesize that the antagonism of neurokinin -1 receptor (NK1R), SP's preferred receptor, can suppress nociception, reduce inflammation and promote wound healing following corneal injuries. We aim to study NK1R antagonist topical treatment in two injury models: mechanical and chemical (alkali burn). In aim 1, we focus on the analgesic efficacy of NK1R antagonism and its effect on corneal nerves. In aim 2 we explores the anti-inflammatory properties of NK1R antagonism and its effect on corneal wound healing.

2. KEYWORDS:

Ocular Pain, Substance P (SP), Neurokinin-1 Receptor (NK1R) Antagonist, Mechanical Injury, Alkali Burn, Nociception, Eye-Wiping Test (EWT), Palpebral Ratio (PR) Nerve Regeneration, Neuroinflammation, Wound Healing

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Task 1. Evaluate the efficacy of NK1R antagonism in suppressing nociception

1.1.Clinical assessment of eyes treated with NK1R antagonist as anti-nociceptive.

Milestone/target date: 03/2024

- Ongoing (70%)

1.2. Evaluation of NK1R antagonism on pain-associated neuron activity and molecular markers

Milestone/target date: 03/2024

- Ongoing (25%)

Major Task 2. Evaluate the efficacy of NK1R antagonism on suppression of inflammation and fibrosis

2.1. Clinical assessment of NK1R antagonism as an anti-inflammatory

Milestone/target date: 09/2025

- Not started

2.2. Cellular and molecular analysis of the effect of NK1R antagonism on inflammation, edema and fibrosis

Milestone/target date: 09/2025

- Not Started

What was accomplished under these goals?

Major activities:
We aimed to explore the analgesic effect of NK1R antagonism following mechanical and chemical (alkali burn) corneal injuries and the related molecular changes. Immediately after injury, mice received a topical NK1R antagonist or vehicle as treatment 2x daily. Clinical assessment has been completed by performing eye-wiping test (EWT) and measuring the palpebral ratio (PR). SP expression in the injured corneas and ipsilateral trigeminal ganglions (TG) was measured by ELISA. Our findings were summarized in two abstracts presented at ARVO 2023 titled: 1) NK1R Inhibition: a Novel Ocular Pain Management Strategy Following Mechanical Corneal Injury, 2) Effect of Neurokinin 1 Receptor Antagonist on Pain Response in Corneal Alkali Burn.

Specific Objectives:
- **Subtask 1.1. Clinical assessment of eyes treated with NK1R antagonist as anti-nociceptive.**
- **Subtask 1.2. Evaluation of NK1R antagonism on pain-associated neuron activity and molecular markers**
- **SubAim 2.1. Clinical assessment of NK1R antagonism as an anti-inflammatory**
- **Subtask 2.2. Cellular and molecular analysis of the effect of NK1R antagonism on inflammation, edema and fibrosis**

Significant results:
Subtask 1.1 Clinical assessment of eyes treated with NK1R antagonist as anti-nociceptive.
Mechanical Injury: Mice were injured by removing corneal epithelium and partial anterior stroma using Algerbrush II. EWT was performed by instillation of hypertonic saline (2M NaCl) on days 0, 4, 7, 14, and 21 to evaluate hyperalgesia. At the same time, an automated video analysis was used to assess allodynia by quantifying PR of the animals' eyes after instillation of iso-osmolar saline (0.9% NaCl, i.e. 0.15M) as innocuous stimulus. L-733,060 (1µg/µl), an NK1R antagonist, or its PBS vehicle was administered topically twice daily immediately
after injury until day 21. One-way ANOVA followed by Tukey's post hoc test or student t-test was used to evaluate the difference among experimental groups. Results are summarized in **Fig. 1.**

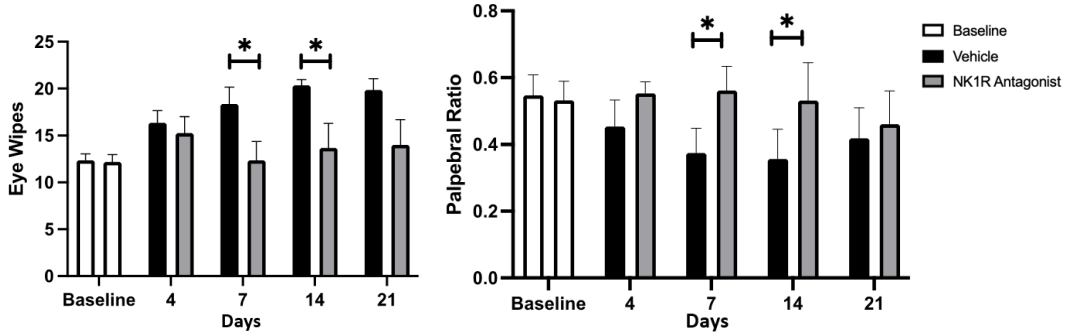

Fig. 1 NK1R Antagonism Reduces Pain Perception following Mechanical Injury
Injured mice developed worsening hyperalgesia (assessed by eye-wiping test) and allodynia

(assessed by palpebral ratio). Both hyperalgesia and allodynia were significantly reduced in L-733,060 treated group compared to the vehicle group on days 7 ($P<0.05$) and 14 ($P<0.05$). n = 6/group. All data are presented as means ± standard deviation. *P < 0.05 (Student t-test).

Chemical Injury: Corneal alkali burn was induced in mice by placing a paper disc (2-mm diameter) soaked in 1 N NaOH for 10 seconds on the central cornea. 6 mice with 1:1 sex ratio were used for each experiment. Animals were randomized into two groups receiving L-733,060 (1μg/μl) or PBS, topically twice a day for 28 days after alkali burn. On days 4, 7, 14, 21 and 28, EWT was performed to evaluate hyperalgesia after applying hypertonic saline (2M NaCl) as noxious stimulus. Following EWT, allodynia was assessed by quantifying PR via an automated video analysis of the animals' eye after instillation of a saline solution iso-osmolar to tears (0.15M NaCl) as innocuous stimulus. Results are summarized in **Fig. 2.**

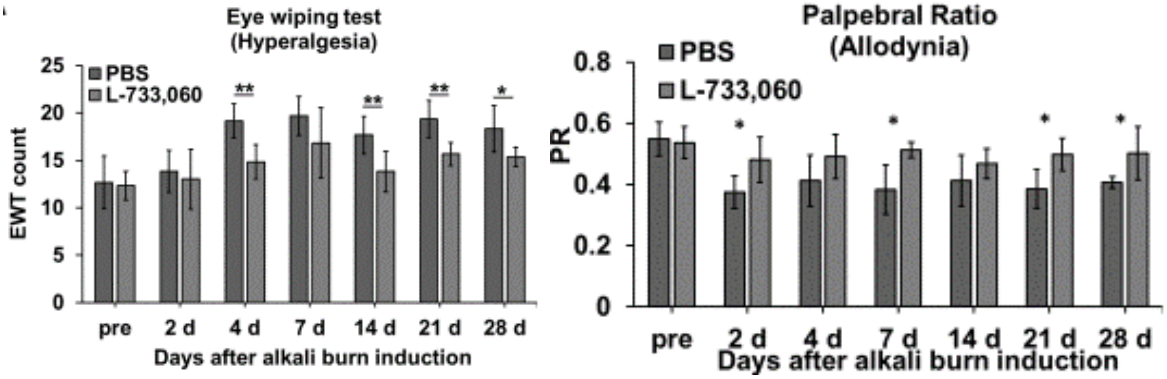

Fig. 2 NK1R Antagonism Reduces Hyperalgesia and Allodynia following Corneal Alkali Burn. NK1R antagonism (L-733,060) significantly reduced hyperalgesia (measured by the EWT) on days 4, 14, 21, and 28, and reduced allodynia (measured by PR) on days 2, 7, 21, and 28 following alkali burn injury. n = 6/group. All data are presented as means ± standard deviation. *P < 0.05, **P < 0.01 (Student t-test).

Subtask 1.2. Evaluation of NK1R antagonism on pain-associated neuron activity and molecular markers

Mechanical Injury: SP expression in the injured corneas and ipsilateral trigeminal ganglions (TG) was measured by ELISA. Corneal SP expression levels persistently increased after the injury **(Fig 3).** SP levels were reduced locally in the cornea, but not centrally in the TG in the NK1R antagonist-treated group **(Fig. 4).**

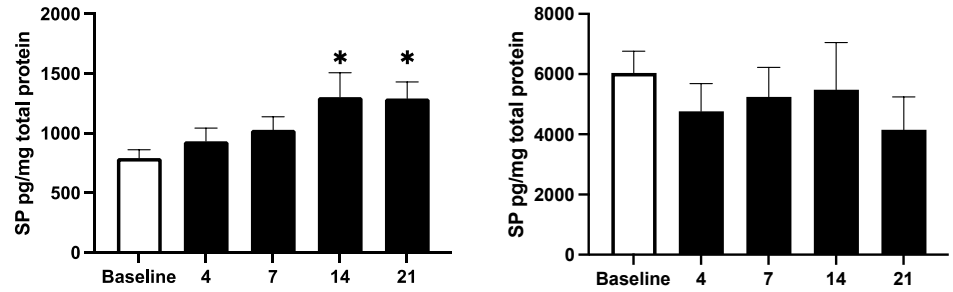

Fig. 3 SP Levels Increase following Mechanical Corneal Injury
Corneal SP expression levels (left panel) persistently increased from 7,921 ± 703 pg/mg before injury to 13,040 ± 2,027 pg/mg on day 14 ($P<0.05$) and 12,927 ± 1,383 pg/mg on day 21 ($P<0.05$) after the injury. No major changes were observed in the TG (right panel). n = 6/group. All data are presented as means ± standard deviation. *P < 0.05 (Student t-test)

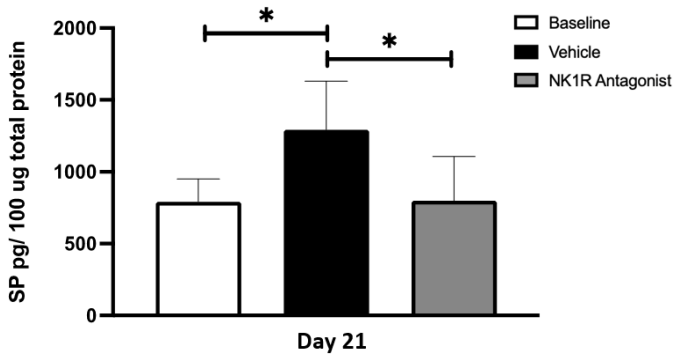

Fig. 4 NK1R Antagonism Reduces SP Levels in Injured Cornea
On day 21, SP levels in the L-733,060 treated group were reduced locally in the cornea compared to the vehicle group (-25.7%). n = 6/group. All data are presented as means ± standard deviation. *P < 0.05 (Student t-test)

Chemical Injury: SP protein levels in the cornea and trigeminal ganglion (TG) were measured by ELISA at days 4, 7, 14, 21, and 28 after corneal alkali burn injury. Compared with normal corneas (shown as "pre" in the figures), alkali burned corneas presented an increase in SP level. The SP levels in TG displayed no significant changes after the injury (**Fig. 5**).

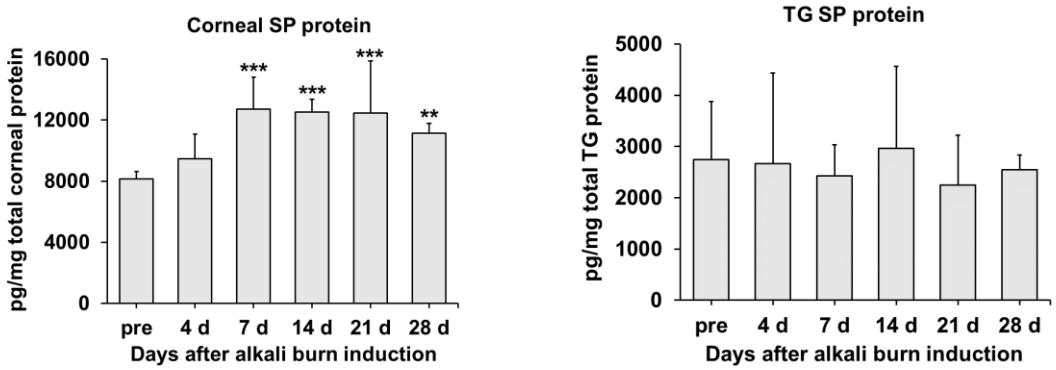

Fig. 5 Locally Increased SP in the Cornea following Corneal Alkali Burn
Compared with normal uninjured corneas (shown as "pre"), alkali-burned corneas presented an increase in SP level on days 7, 14, 21, and 28 days after alkali burn injury. Meanwhile, the SP levels in TG displayed no significant change at any time point after alkali burn. n = 6/group. All data are reported as means ± standard deviation. **P < 0.01, ***P < 0.001 (vs. pre, Student t-test).

Conclusions: Mechanical and alkali burn corneal injuries increase the SP levels in the cornea. Our data suggest that SP is a key mediator of nociception after corneal injury. Suppression of its function via inhibition of its receptor NK1R reduces hyperalgesia and allodynia in both the mechanical and chemical injury models. Thus, topical treatment with NK1R antagonist leads to a decrease in nociception and shows promise for the treatment of ocular pain.

State goals not met:

SP is a key mediator of corneal nociception which involves peripheral (corneal) and afferent (TG) components. Originally, we hypothesized that SP expression would increase in the TG as well as in the cornea. However, the SP levels in TG showed no significant change in either model. A plausible explanation for this observation might be the death of the ganglion, since previously axotomy was shown to be associated with ganglion death and decreased neuropeptide production in other animal studies.

Our team yet has to perform IVCM on these animal models to elucidate the effect of NK1R antagonism on neuroregeneration. We intend to perform those studies as part of Subaim 2.1 since we believe that NK1R antagonism's anti-inflammatory effects are what permit neuroregeneration. The data of molecular changes taking place in the TG following corneal injury, specifically the transcriptomic changes, are still being processed and hence not included in this 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?

During the next reporting period, we would like to (1) focus on cellular activity and molecular markers of TG neurons following chemical and mechanical injury to assess the analgesic potential of NK1R antagonism, (2) determine the clinical efficacy of NK1R antagonism in supporting nerve regeneration, reducing corneal neovascularization, scarring and thus promoting would healing after mechanical and chemical injuries and (3) determine the antinflammatory properties of NK1R antagonist.

4. IMPACT:

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

1) We have established that elevation of SP in cornea is correlated with pain sensation in corneal injuries.

2) We have established the analgesic effect of inhibiting SP receptor- NK1R in the treatment of ocular pain following mechanical and chemical corneal injuries.

3) Reduction of SP levels in injured cornea suggests a negative feedback loop by topical NK1R antagonism and the potential mechanism for NK1R analgesic efficacy.

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:

Nothing to Report.

Changes in approach and reasons for change

Nothing to Report.

There were no significant changes in the project or its direction during this reporting period.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report.

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

No significant changes in use of biohazards and/or select agents.

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.

2 Abstracts have been presented to *ARVO 2023*:

1)*Neurokinin 1 Receptor Antagonist: a Novel Analgesic for Pain following Corneal Alkali Burn.* Hideaki Someya, Amirreza Naderi, Aytan Musayeva, Francesca Kahale, Akitomo Narimatsu, Yihe Chen, Reza Dana
Acknowledgement of federal support (yes).

2) *NK1R Inhibition: a Novel Ocular Pain Management Strategy Following Mechanical Corneal Injury.* Pier Luigi Surico, Amirreza Naderi, Hideaki Someya, Aytan Musayeva, Francesca Kahale, Yihe Chen, Reza Dana
Acknowledgement of federal support (yes).

- **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:	Dr. Reza Dana
Project Role:	*PI*
Researcher Identifier:	
Nearest person month worked:	0.82 Calendar Months
Contribution to Project:	*PI supervises all aspects of the project, including technical training of postdoctoral fellow, experimental design, troubleshooting, and data analysis. This also includes holding meetings with fellow (once/week) to discuss progress and direction of the project.*
Name:	Dr. Yihe Chen
Project Role:	*Co-PI*
Researcher Identifier:	
Nearest person month worked:	0.94 Calendar Months
Contribution to Project:	*PI supervises all aspects of the project, including technical training of postdoctoral fellow, experimental design, troubleshooting, and data analysis. This also includes holding meetings with fellow (once/week) to discuss progress and direction of the project.*
Name:	Ann Yung
Project Role:	*Pre-doctoral Research Fellow*
Researcher Identifier:	
Nearest person month worked:	*2.71 Calendar Months*
Contribution to Project:	*Under direct supervision of PI/Co-PI, Ann Yung performs the experiments described in this project.*
Name:	Amirreza Naderi
Project Role:	*Pre-doctoral Research Fellow*
Researcher Identifier:	

Nearest person month worked: 10 Calendar Months
Contribution to Project: *Under direct supervision of PI/Co-PI, Ann Yung performs the experiments described in this project.*

Name: *Liangju Kuang*
Project Role: *Postdoctoral Researcher*
Researcher Identifier:
Nearest person month worked: 6 Calendar Months
Contribution to Project: *Under direct supervision of PI/Co-PI, Dr. Kuang performs the experiments described in this project.*

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

The following has been changed for the PIs since the last submission:

Dr. Reza Dana

1. This grant ended: W81XWH-18-1-0654 (09/01/2018-08/31/2022) – "Novel Adhesive Biomaterials for Quick and Long-Lasting Sutureless Repair of Corneal Injuries"
2. This grant ended: R21EY032695 (05/01/2021-03/31/2023) – "Therapeutic Function of alpha-Melanocyte Stimulating Hormone (α-MSH) in Acute Injury and Chronic Degeneration of Corneal Endothelium"

Dr. Yihe Chen

1. This grant ended: R21EY031781 (05/01/2021-06/30/2023) – "Pathogenicity of memory Th17 cells in chronic autoimmune uveitis"
2. This grant was awarded: R01EY034111 (05/01/2023 – 04/30/2028) – "Mechanisms of immunological memory-mediated pathogenesis in chronic autoimmune uveitis"
3. This grant was awarded: W81XWH-22-1-0889 (09/30/2022-09/29/2025) – "Safety and Efficacy of Supersaturated Oxygen Emulsion (SSOE) as a Novel Topical Treatment in Open Globe Injury"
4. This grant was awarded: Massachusetts Lions Eye Research Fund (09/01/2022-02/29/2024) – "Hypoxia-inducible factor-1 mediated regulation of memory T cells in chronic uveitis"

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

.