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TITLE: Targeting the CRMP2-Ca2+ Channel Complex for Abortive Treatment of Migraine and Post-Traumatic Headache

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14. ABSTRACT

Migraine is one of the world's most common neurological disorders. Current acute migraine treatments have sub-optimal efficacy and new therapeutic options are needed. Approaches targeting calcitonin gene related peptide (CGRP) signaling are clinically effective but small molecule antagonists have not been advanced due to toxicity. In this study, we explored the axonal growth/specification collapsin response mediator protein 2 (CRMP2) as a novel "druggable" target for inhibiting CGRP release and for potential relevance for treatment of migraine pain and post-traumatic headache. CRMP2 has been demonstrated to regulate N-type voltage gated Ca2+ channel (CaV2.2) activity and Ca2+-dependent CGRP release in sensory neurons. The co-expression of CRMP2 with CaV2.2 and CGRP in trigeminal ganglia (TG) sensory neurons suggested the possibility of a novel approach to regulate CGRP release in the trigeminal system. Screening protocols surprisingly revealed that (S)-Lacosamide ((S)-LCM), an inactive analog of the clinically-approved small molecule anti-epileptic drug (R)-Lacosamide (Vimpat®), inhibited CRMP2 phosphorylation by cyclin dependent kinase 5 (Cdk5) in rat TG slices and decreased depolarization-evoked Ca2+ influx in TG cells in culture. We found that (S)-LCM inhibited nitric oxide (NO)-donor-induced allodynia in a rat model with triptan-induced latent sensitization, demonstrating its potential in mitigating migraine. Furthermore, we refined and optimized a weight drop mouse model of mild traumatic brain injury (mTBI) and characterized the persistent post traumatic headache (PPTH) phase when animals no longer have allodynia but have increased vulnerability to experience pain from normally subthreshold stimuli.

15. SUBJECT TERMS

migraine, CRMP2, Cav2.2, (S)-Lacosamide, allodynia, capsaicin-evoked CGRP release, cranial cup, inflammatory mediators, nitric oxide donor, sodium channels, calcium channels, traumatic brain injury, post traumatic headache excitability

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Table of Contents

1. **INTRODUCTION:**

Migraine affects approximately 11-13% of the adult population in the United States and is a prevalent and under-diagnosed disorder that severely impacts quality of life of afflicted individuals and presents an enormous economic cost to society. Soldiers have a three-fold higher incidence of migraine than civilians[1,2]. Post-traumatic headache (PTH) often presents with a migraine phenotype and affects upwards of 97% of all soldiers with traumatic brain injury (TBI). Acute treatments are used to relieve pain and associated symptoms, and commonly include triptans and nonsteroidal anti-inflammatory drugs (NSAIDs). However, current treatments for the PTH and migraine are often inadequate, fewer than one-half of patients taking oral triptans report being pain-free at 2 hours, and up to one-third report headache recurrence within 24 hrs[3,4]. The response rate for NSAIDs is similar[5-7]. Therefore, it is significant to develop new treatments to address the unmet medical need for migraine and PTH in military personnel. It has been demonstrated that calcitonin gene related peptide (CGRP) plays a cardinal role in migraine headache[8]. Blood levels of CGRP are elevated during migraine attacks[9]. It is likely that CGRP does not directly activate trigeminal dural afferents but potentiates the release of nociceptive agents into the perivascular space, possibly from mast cell degranulation that has been proposed to contribute to migraine[10,11]. We have previously demonstrated that the axonal growth/specification collapsin response mediator protein 2 (CRMP2) regulates CaV2.2 activity and Ca^{2+}-dependent CGRP release in sensory neurons. CRMP2 levels are increased in chronic migraine patients and decreasing CGRP activity has been clinically validated for migraine therapy[12]. Our previous work demonstrated that (*S*)-Lacosamide ((*S*)-LCM) inhibits CRMP2 phosphorylation, inhibiting CaV2.2-CRMP2 association and CaV2.2 activit[13]. (*S*)-LCM is an enantiomer of the clinically approved anti-epileptic drug Vimpat®, (*R*)-LCM and was shown to dock well to CRMP2. Taken together, modulation of trigeminal dural afferents, or of their post-synaptic pathways, or both, can provide effective therapy for migraine and PTH. We hypothesize that (*S*)-LCM, through inhibition of Cdk5-mediated phosphorylation of CRMP2, inhibits CaV2.2 activity and consequently diminishes CGRP release. By limiting CGRP release, which is increased in the jugular blood of migraineurs during attack[14], (*S*)-LCM will be an effective abortive treatment for migraine and PTH. The goal of this study was to determine if (*S*)-LCM is effective in preclinical models of migraine and PTH by inhibiting interactions of CRMP2 and N-type calcium channels providing a rationale for advancement to human trials. **KEYWORDS:** migraine, traumatic brain injury (TBI), post traumatic headache (PTH), collapsin response mediator protein 2 (CRMP2), Cav2.2, allodynia, (*S*)-Lacosamide (LCM), calcitonin gene related peptide (CGRP) release, cranial cup, inflammatory mediators, nitric oxide (NO) donor, voltage-gated sodium channels, voltage-gated calcium channels, excitability, constellation pharmacology

2. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

In this project, we proposed to test the hypothesis that (*S*)-LCM inhibits voltage-gated calcium channel CaV2.2 activity and consequently diminishes CGRP release providing a strong rationale for treatment of abortive migraine and post-traumatic headache (PTH). By limiting CGRP release, which is increased in the jugular blood of migraineurs during attack, (*S*)-LCM will be an effective abortive treatment for migraine and PTH. Decreasing CGRP activity has been clinically validated for migraine therapy, and we have previously found that the axonal growth/specification CRMP2 regulates CaV2.2 activity and Ca^{2+}-dependent CGRP release in sensory neurons and that (*S*)-LCM) has preferential activity on Ca^{2+} channels through the modulation of CRMP2 phosphorylation. While (*S*)-LCM has not yet undergone clinical evaluation, it likely allow rapid advancement and evaluation of this molecule to humans based on drug-like qualities and safety of the enantiomer, (*R*)-LCM[15]: long half-life (3 h), 100% oral bioavailability up to 20 mg/kg with 40 min peak concentration, optimum brain to plasma partition coefficient (0.55).

The major goal of this project was to determine if (*S*)-LCM is effective in preclinical models of migraine by inhibiting interactions of CRMP2 and N-type calcium channels providing a rationale for advancement to human trials for migraine and for PTH.

Studies in the Porreca lab focused on evaluating the efficacy and duration of action of (*S*)-LCM in abolishing IM-induced CA in preclinical models, dural inflammatory mediator (IM)- and nitric oxide (NO) donor-induced models, and the effects on CGRP levels from jugular blood, a translational biomarker for migraine (Aim 2). It was also proposed to characterize the presence of CA in a model of repetitive mild traumatic brain injury (mTBI) and evaluate the possible efficacy of (*S*)-LCM in this model (Aim 3)

In our application, the following aims were proposed to be completed at the Khanna and Porreca Laboratories during the entire funding period:

- **<u>Aim 1 (Khanna lab)</u>: Efficacy and mechanism of (*S*)-LCM in blocking Ca^{2+} currents in cultured TG neurons innervating dura from rats.** In this Aim, we will confirm the mechanism of action of (*S*)-LCM by investigating ionic changes in TGs from vehicle or sumatriptan-treated rats and on TGs from mice subjected to sham injury or to repetitive mild TBI in order to explore changes that may be relevant to "migrainous" biology.
    - **Aim 1.1**. Assessing inhibition of Cdk5-mediated phosphorylation of CRMP2 in TGs that innervate the dura mater.

- **Aim 1.2**. Assess the inhibition of CRMP2-CaV2.2 association in these TG cells.
- **Aim 1.3**. Test the inhibition of Ca^{2+} currents by (*S*)-LCM in cultured identified dural TG neurons.
- **Aim 1.4**. Test the effects of (*S*)-LCM on excitability of TG neurons.
- **Aim 1.5**. Perform a phenotypic screening of TG neurons innervating the dura mater utilizing the "constellation pharmacology" paradigm

- **<u>Aim 2 (Porreca Lab)</u>: Determine if (*S*)-LCM prevents periorbital and hindpaw cutaneous allodynia (CA) and blood CGRP elevation in rats.**
- Determine if (*S*)-LCM prevents periorbital and hindpaw CA and blood CGRP elevation in rats. In this aim, the in vivo efficacy of (*S*)-LCM in inhibiting CA and CGRP plasma levels will be evaluated in 2 cephalic pain models using two different routes at multiple time points in male and female rats.
  - **Aim 2.1**. Does (*S*)-LCM abolish the development of CA induced by IM in rats?
  - **Aim 2.2**. Determine if (*S*)-LCM prevents NO donor-induced CA and blood CGRP elevation in rats with triptan-induced latent sensitization.
- **<u>Aim 3 (Porreca Lab)</u>: Assessing cephalic pain and efficacy of (*S*)-LCM in a repetitive mTBI rodent model.** The goal of this aim is to characterize the presence and neurochemical characteristics of PTH following repetitive mTBI in mice as a model of PTH and to evaluate efficacy of (*S*)-LCM to reverse CA.
  - **Aim 3.1.** Assess the efficacy of (*S*)-LCM in abolishing TBI-induced allodynia in mice.
  - **Aim 3.2** Assess the possible presence of ongoing headache in mice with repetitive mTBI.
  - **Aim 3.3.** Determine the consequences of repetitive mTBI on the function of nerves innervating the dura mater using the cranial cup preparation. Detect the release of CGRP by dural afferents in TBI mice and determine the effect of (*S*)-LCM.

- **<u>Aim 4 (KHANNA lab)</u>: Does (*S*)-LCM have significant adverse effects at therapeutic doses for treatment of cephalic pain and PTH?**
  - **Aim 4**. Assess the potential liabilities of (*S*)-LCM preclinically as a step in advancement to humans using a battery of widely accepted rodent models.

- **What was accomplished under these goals?**

1) Major activities: During this funding period, we have performed the majority of experiments proposed for Aim 3.

2) Objectives: Our objectives during this period were to establish the mTBI model and stress-related PTH following repetitive mTBI and evaluate efficacy of (*S*)-LCM in these models.

3) Significant results: We have expended considerable effort in setting up and characterizing the mouse model of mTBI-induced PTH. We showed that the model largely recapitulates the effects of a mTBI in humans (see below). We showed that following mTBI, there was a period of transient allodynia reminiscent of PTH and after these changes in sensory threshold were resolved, exposure of mTBI-treated mice to a subsequent provocative challenge reinstated the cutaneous allodynia, reminiscent of persistent PTH (PPTH); exposure to bright light stress (BLS) was chosen as the provocative "second hit" stimulus, replicating the two-hit hyperalgesic priming concept that has been used in pain research. For the model we modified a previously reported weight drop model[16] that is performed in unrestrained mice and allows free motion of the head that imparts linear and rotational acceleration in the brain[17,18]. The biomechanical forces mimic a typical human mTBI (Fig 10B)[19]. In accordance with the clinical classification of mTBI the injury causes no skull fractures, no seizures, no cavitation, no lesion or significant neuronal loss as confirmed by MRI but does induce a brief delay in sensory responsiveness (i.e. righting reflex). A limitation of this model is that animals receive the mTBI impact while under light anesthesia with isoflurane. In humans, migraine patients report sensitivity to light and touch during and even between headache attacks. Studies in TBI patients are lacking, but similar to migraine patients in our mTBI model, male or female mice acutely displayed increased sensitivity to normally innocuous mechanical stimuli (i.e., allodynia) in both cephalic (Figures 10D and 11E) and extra-cephalic (Figure 11F) regions that resolves after mTBI. The period of sustained allodynia is reminiscent of acute PTH in patients. In the mouse model we also demonstrated that botox, an approved medication for patients with frequent migraine, showed prevention of stress-induced cephalic allodynia helping in validating the mouse model of mTBI-induced PTH. Additionally, once the mTBI-induced allodynia resolved, we demonstrated that we could re-instate cutaneous allodynia with exposure only of mTBI animals to a period of bright light stress (15 min, unrestrained in their home cages).

Methods and results for Aim 3: Efficacy of (S)-LCM in cephalic pain from repetitive mild traumatic brain injury (rmTBI) in mice.

For a mTBI model, we modified a previously reported weight drop model[16] that is performed in unrestrained mice and allows free motion of the head that imparts linear and rotational acceleration in the brain[17,18] (Figure 10B). Briefly, male C57BL/6J mice were acclimated to the von Frey chambers daily for 5 days of 3 h/day. After baseline of periorbital and hindpaw tactile threshold, the animals received a 100 g weight dropped from 100 cm above the skull to the top of the head between bregma and lambda once under light anesthesia with isoflurane. In accordance with the clinical classification of mTBI the injury did not cause skull fractures, seizures, cavitation, lesion or significant neuronal loss as confirmed by MRI but induced a brief delay in sensory responsiveness. Mice spontaneously recovered the righting reflex after some delay and showed no evidence of seizures, paralysis or impaired behavior. The cutaneous allodynia was measured at various time points as the surrogate marker of PTH.

**A mouse model of mTBI (1st hit) was set-up for PTH studies, and in the model male or female mice acutely displayed increased sensitivity to normally innocuous mechanical stimuli (i.e., allodynia) in cephalic region resolved by post-mTBI. (Figure 10C) The period of sustained allodynia is reminiscent of the headache after recovering consciousness in patients. After returning to baseline, exposure to BLS (2nd hit) provoked cephalic CA in mTBI mice suggesting a development of a state of central sensitization. (Figure 10D)*

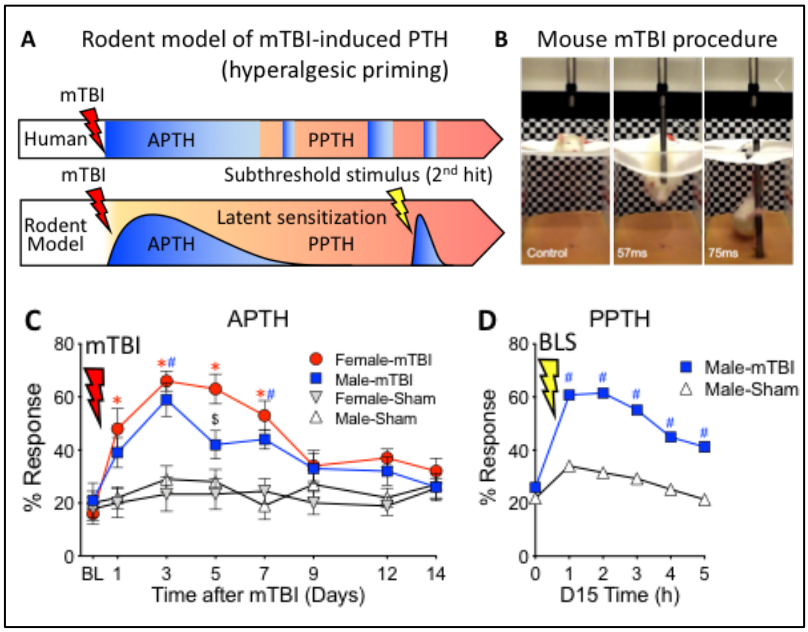

Figure 10. Mouse model of acute and persistent PTH. (A) In patients (top), following the headache after recovering consciousness (i.e., acute PTH, APTH) phase many patients transition to a persistent PTH (PPTH) phase where they experience frequent headaches that appear to be related to headache triggers. We modeled PTH and PPTH in mice (bottom) using a two-hit model of hyperalgesic priming with mTBI (1st hit, red icon) resulting in PTH characterized by transient cephalic allodynia and long-lasting latent sensitization. During the latent sensitization period, a second hit (yellow icon) reinstated cephalic allodynia, reminiscent of PPTH. (B) Weight drop model of mTBI. (C) Periorbital allodynia was observed in male and female mice for 14 days following mTBI. (D) When tactile responses return to baseline, exposure to BLS elicited allodynia selectively in mTBI but not sham mice.

- **Summary**:
  This funding period has allowed us to establish a mouse model of mTBI and characterize the late PTH phase when animals no longer have allodynia but have increased vulnerability to experience pain from normally subthreshold stimuli.

- **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?**

  Nothing to Report.

3. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*
   - **What was the impact on the development of the principal discipline(s) of the project?**

     Our previous work demonstrated the efficacy of (*S*)-LCM in mitigating cephalic and extracephalic allodynia in two different migraine models triggered by direct dural activation (IM or NO donor). Pre-treatments of (*S*)-LCM via p.o. or i.p. were very effective in inhibiting IM-induced allodynia compared to post-treatment suggesting higher potential as a prophylactic therapy.
     The major accomplishment of this funding period was the establishment and characterization of a mouse model of mTBI for PTH and for PPTH. We adapted, refined and optimized a weight drop mouse model of mTBI in male and female mice of two different strains to characterize both the early PTH and, following resolution of initial mTBI-induced pain, a persistent PTH (PPTH) phase when animals have baseline sensory thresholds but show increased vulnerability to pain from normally subthreshold stimuli. In the model, resulting cephalic and extracephalic allodynia was well characterized as well as latent sensitization to BLS. One application of the mTBI caused long-lasting sensitization of the animals to BLS even after the initial cephalic pain resolved suggesting relevance to mechanism of PPTH.

   - **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.

4. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
   - **Changes that had a significant impact on expenditures**
   - **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**
   - **Significant changes in use or care of vertebrate animals.**
   - **Significant changes in use of biohazards and/or select agents**

     Nothing to Report

5. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*
   - **Publications, conference papers, and presentations**
     - **Journal publications.**
     - **Books or other non-periodical, one-time publications.**
     - **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.

6. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   - **What individuals have worked on the project?**

Name:	**Frank Porreca**
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-2267-0617
Nearest person month worked:	2
Contribution to Project:	Dr. Porreca advised the whole team and provided oversight for the entire project.
Funding Support:	NA
Name:	**Yeon Sun Lee**
Project Role:	Senior Scientist
Researcher Identifier (e.g. ORCID ID):	0000-0002-0472-4765
Nearest person month worked:	6
Contribution to Project:	Dr. Lee designed the study, coordinated the workflow, analyzed the data and wrote the report.
Funding Support:	NA
Name:	**Chaoling Qu**
Project Role:	Research Scientist
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	8
Contribution to Project:	Ms. Qu executed the experiments and collected the behavioral data.
Funding Support:	NA

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

- **Organization Name:**
- **Location of Organization:**
- **Partner's contribution to the projec**t*)*
  - **Financial support;**
  - **In-kind support**;
  - **Facilities**;
  - **Collaboration**;
  - **Personnel exchanges**
  - **Other.**

Nothing to Report.

7. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:**

Two separate reports for Drs. Khanna and Porreca have been submitted to https://ers.amedd.army.mil for each award.

- **QUAD CHARTS:**

NA

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APPENDICES:

None.