The (R)- isomer of isometheptene decreases trigeminal sensitivity in a rat model of primary headache

Jessica Perino, MS[1], Nathan T. Fried, PhD[2], Michael L. Oshinsky, PhD[3], Bruce Daugherty, PhD, MBA[4], Seth Lederman, MD[4], Melanie B. Elliott, PhD[1,2]

[1]Department of Neurosurgery; [2]Department of Neuroscience; [3]Department of Neurology, Thomas Jefferson University, [4]Tonix Pharmaceuticals Holding Corp

Introduction

Isometheptene is thought to be the active ingredient of the commonly known headache medication, Midrin. Previously, we found only the (R) enantiomer to be effective in alleviating headache behavior in two models of headache compared to the (S) enantiomer.

Experiments using electrical and mechanical stimulation of the dura in animals with no history of head pain have been used to elucidate the mechanisms of the trigeminovascular system at the level of the trigeminal ganglion, trigeminal nucleus caudalis, periaqueductal gray, and the thalamus. The limitation of these acute models is that electrical and mechanical stimulation of the dural blood vessels does not cause long lasting pain similar to recurrent headache pain in humans. Two animal models for the investigation of headache pathophysiology that include aspects of the chronic nature of headache were included in this study. These models provide a new approach to study the pathophysiology of recurrent headache and support validation of novel treatments.

Using behavior methods of monitoring trigeminal allodynia in rats, our group discovered a rat with spontaneous episodic trigeminal allodynia (STA) (Oshinsky et al, 2012). The model was established through 18 generations of inbreeding for testing analgesics and the further understanding of mechanisms of migraine. STA rats experience similar symptoms to human migraine patients, such as episodic trigeminal sensitivity, phonophobia, responsiveness to abortive and prophylactic headache treatments, and sensitivity to migraine triggers. A second well-characterized model of repeated dural inflammatory stimulation (IS), in which an inflammatory stimulant is infused over the dura, also results in persistent headache behavior that has been accepted as a valid model for drug testing. Although the pathological mechanisms between the two models are different, one being considered idiopathic or a spontaneous model and the other inflammatory in nature, the precise mechanism(s) remain elusive.

The aim of this study is to determine the dose response of the (R)-isomer of isometheptene on trigeminal sensitivity in the STA and IS rat models and study the mechanism of action of isometheptene.

Methods

Experimental Design

To determine the efficacy of the R-isomer of isometheptene in the two rat trigeminal headache models, a 4 point dose response curve was conducted.

Periorbital thresholds, measured using von Frey filaments, were obtained to determine trigeminal sensitivity prior to and at 0.5 hr-, 1.5 hr-, 2.5 hr-, 3.5 hr-, and 24 hr-post-treatment with either (R)-isometheptene (1, 10, 30, 60 mg/kg), or saline vehicle. A 100 mg/kg dose was omitted due to mucate salt related fatalities observed in the rat models, though it was well tolerated in human testing. All treatments were administered intraperitoneally. Von Frey data was analyzed using Prism, and a Two-Way ANOVA was performed to determine statistical significance with the factors group and time.

Following the completion of the 24 hr-post treatment time point, animals were perfused with 4% PFA. Trigeminal ganglion and brainstem were collected and sectioned at 20µm. Subsequently, immunostaining was performed to assess the changes in levels of two nociceptive signaling molecules, calcitonin gene-related peptide (CGRP) and neuronal nitric oxide synthase (nNOS). Standard immunofluorescence protocols used previously were followed for the staining of CGRP and nNOS (Diautolo et al, 2016) .

Model Generation

The STA animals selected for this study represented the 17-20th generation of inbreeding and exhibited the spontaneous trigeminal allodynia trait. Animals were trained to enter restraint freely in order to perform von Frey and establish periorbital baseline.

The IS animals were surgically generated through cranial cannula implantation. This allowed for repeated dura stimulation that leads to sensitization of the trigeminovascular afferents and secondary order neurons in the brainstem (Oshinsky et al, 2007). The rats received up to 15 infusions of prostaglandin onto the dura in order to transition to a lower trigeminal von Fry threshold. Following the completion of infusions, the animals undergo one week with no behavior testing or infusions, followed by a week of behavior testing to determine if the animal is chronically sensitive.

Results

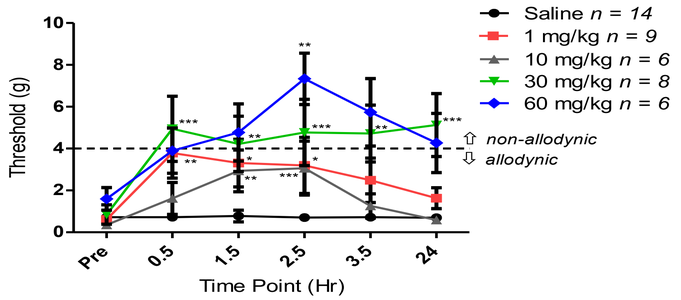

Figure 1: **R-isomer of the isometheptene (ISO) dose response in the STA Model.** STA rats showed a significant increase in trigeminal threshold in the 1 mg/kg of ISO treatment at the 0.5 hr (**P<0.01), 1.5 hr (*P<0.05), and 2.5 hr (*P<0.05). Testing of 10 mg/kg of ISO showed increases in thresholds at 1.5 hr (**P<0.01) and 2.5 hr (***P<0.001). Testing of 30 mg/kg of ISO showed increases in trigeminal thresholds at the 0.5 hr (***P<0.001), 1.5 hr (**P<0.01), 2.5 hr (***P<0.001), 3.5 hr (**P<0.01), and 24 hr (***P<0.001). Testing of 60 mg/kg of ISO showed increases in trigeminal thresholds at the 1.5 hr (*P<0.05), 2.5 hr (****P<0.001), and 3.5 hr (**P<0.001).

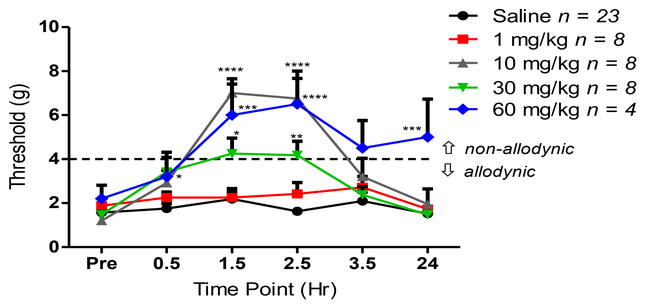

Figure 2: R-isomer of the isometheptene (ISO) dose response in the IS Model. Treatment with 10 mg/kg of ISO showed increases in trigeminal thresholds at the 1.5 hr (****P<0.0001) and 2.5 hr (****P<0.0001). Administration of 30 mg/kg of ISO resulted in increases in trigeminal thresholds at the 1.5 hr (*P<0.05) and 2.5 hr (**P<0.01). Testing of 60 mg/kg of ISO showed increases in trigeminal thresholds at the 1.5 hr (***P<0.001), 2.5 hr (****P<0.0001) and 24 hr (***P<0.001).

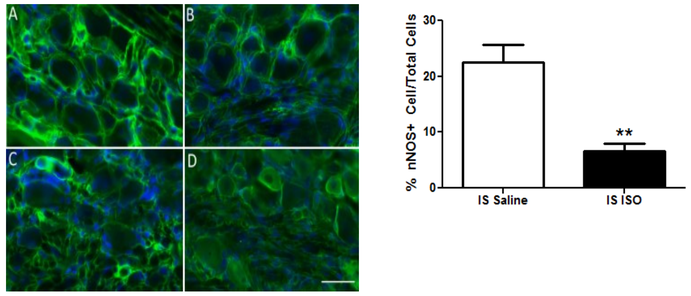

Figure 3: Trigeminal ganglia nNOS immunostaining. Green staining indicates nNOS, blue represents DAPI staining. V1 ophthalmic branch was selected for imaging. **A**. IS model saline control. **B**. IS model R-ISO 60mg/kg dose. **C**. STA model saline treatment. **D**. STA model 60 mg/kg testing. Animals were perfused immediately following 24 hr. VF time point. Statistical significance of positively stained nNOS cells was determined by cell counting (**P<0.004) n=4

Figure 4: Trigeminal ganglia CGRP immunostaining. Green staining indicates CGRP, blue represents DAPI staining. V1 ophthalmic branch was selected for imaging. V1 ophthalmic branch was selected for imaging. **A**. IS model saline treatment. **B**. IS model R-ISO 60mg/kg dosage. **C**. STA model saline administration. **D**. STA model 60 mg/kg testing. Animals were perfused immediately following 24 hr. VF time point. Statistical significance of positively stained CGRP cells was determined by cell counting (*P<0.016) n=4

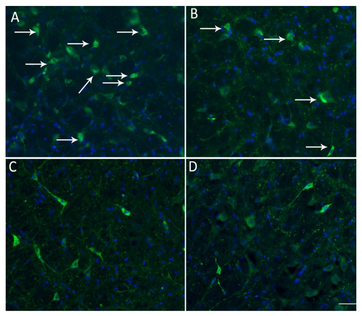

Figure 5: TNC nNOS immunostaining. V1 region was selected for imaging. **A**. IS model saline control. **B**. IS model R-ISO 60mg/kg dose. C. STA model saline treatment. **D**. STA model 60 mg/kg testing. Examples of nNOS positive cells are indicated by arrows.

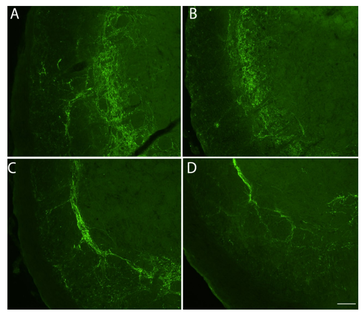

Figure 6: TNC CGRP immunostaining. V1 region was selected for imaging. **A**. IS model saline control. **B**. IS model R-ISO 60mg/kg dose. **C**. STA model saline treatment. **D**. STA model 60 mg/kg testing.

Acknowledgments: Research support was provided by Tonix Pharmaceuticals Holding Corp.
We would also like to thank the Jefferson Headache Center for their support and guidance.
Thank you to Ashley Tyburski for assistance with animal care and valuable input through this project.

Discussion & Conclusions

- Findings show differential dose-response effects of (R)-isometheptene treatment on trigeminal sensitivity that are dependent on the headache model.
- Treatment with (R)-isometheptene showed better efficacy for the IS model at a lower dose compared to the STA model.
- Treatment with (R)-isometheptene in the STA model resulted in a significant improvement in trigeminal thresholds that persisted for 24 hours.
- Side effects at the 60 mg/kg dose included seizures in a subset of animals, possibly affecting the thresholds in this treatment group for both models.
- Treatment with (R)-isometheptene reduced the number of nNOS and CGRP positive trigeminal ganglia neurons and slightly reduced CGRP immunoreactivity in the TNC for both STA and IS models.
- Findings supports the (R)-isomer of isometheptene as a therapeutic for chronic spontaneous type headaches such as migraine, as well as for inflammatory pain conditions.

References:

- Daiutolo BV, Tyburski A, Clark SW, and Elliott MB Trigeminal pain molecules, allodynia, and photosensitivity are pharmacologically and genetically modulated in a model of traumatic brain injury. Neurotrauma. (2016) 33:1–13
- Oshinsky ML, Gomonchareonsiri S. Episodic dural stimulation in awake rats: a model for recurrent headache. Headache. (2007) 47(7):1026-36
- Oshinsky ML, Menka M, Sanghvi MS, Maxwell CR, Gonzalez D, Spangenberg R, Cooper M, and Silberstein S. Spontaneous Trigeminal allodynia in rats: a model of primary headache. Headache. (2012) 52(9): 1336–1349.

Corresponding PI: Melanie.Elliott@jefferson.edu