Introduction

Headache is a highly prevalent symptom in all severities of traumatic brain injury (TBI), and it is one of the most common symptoms of post-traumatic headache. Post-traumatic headache (PTH) remains the most common chronic pain syndrome within the TBI patient population. Mild and moderate forms of TBI are more prevalent than severe forms, but it is currently believed that PTH occurrence is not related to the severity of TBI. Despite being a common symptom following injury, little is known about the pathogenesis of post-traumatic headache. This is partly due to a lack of preclinical animal models studying PTH. A large proportion of mild head injuries are blunt head traumas (i.e., closed head injury) caused by vehicular crashes, falls, sports or recreation activities, and military training regardless of deployment setting. Mild TBI (mTBI) can be a diffuse injury (closed head injury or concussion) or a focal-diffuse injury (e.g. blunt trauma with a lesion).

In many patients, PTH resolves within 3 months of injury; however, in others it can continue for much longer. The International Classification of Headache Disorders defines PTH as headache secondary to head trauma developing within seven days of injury. Headache pain involves abnormal activation of the trigeminovascular system. This can cause the release of calcitonin gene-related peptide (CGRP), a neuropeptide with a known role in migraine. CGRP was also shown to play a role in PTH. CGRP is released in the trigeminal pain pathway by the trigeminal ganglion of cranial nerve V. The release of CGRP has been shown to increase inducible nitric oxide synthase (iNOS) release from trigeminal ganglion glial cells, and a reciprocal relationship between the two is said to exist. The release of iNOS can cause the excessive release of nitric oxide (NO), a damaging free radical. Increases in CGRP have been reversed with sumatriptan, as well as other drugs. Previously, our lab has shown an increase in CGRP in the caudal brainstem after a focal TBI, indicating sensitization of the trigeminal ganglion neurons. The objective of this study was to determine the role of CGRP in iNOS production by employing pharmacological blockade. This study determined the effects of a 5-HT1 receptor agonist (sumatriptan) and a CGRP antagonist (MK8825) on levels of iNOS as these drugs inhibit CGRP release and binding, respectively, on iNOS levels in the ganglia and trigeminal nucleus caudalis.

Results

Figure 1 von Frey allodinia testing: (A) Periorbital thresholds were significantly increased on the ipsilateral and contralateral sides by sumatriptan and MK8825 compared to vehicle, \( p < 0.05 \), \( **p < 0.01 \) and \( ***p < 0.001 \). (B) Forepaw ipsilateral and contralateral thresholds were significantly increased by sumatriptan compared to vehicle, \( **p < 0.01 \) and \( ***p < 0.001 \).

Figure 2 RT-PCR quantification of caudal brainstem iNOS mRNA levels at two weeks after incision, CCI injury treated with vehicle, MK8825, and sumatriptan. iNOS mRNA levels were increased after CCI compared to control, \( p < 0.05 \). iNOS mRNA was decreased after CCI with both MK8825 and sumatriptan treatments compared to CCI mice treated with vehicle, \( *p < 0.05 \) and \( **p < 0.01 \), respectively.

Figure 3 iNOS immunohistochemistry in the trigeminal ganglia: (A) The number of iNOS positive cells at two weeks post-operatively in control, CCI treated with vehicle, CCI treated with MK8825, and CCI treated with sumatriptan. The number of iNOS cells in the vehicle-treated group were increased compared to control, \( ****p < 0.0001 \). iNOS cells were decreased in the MK8825 and sumatriptan groups compared to vehicle, \( ****p < 0.0001 \). (B-F) Images showing immunofluorescent (red) iNOS-labeled ganglia cells. (B) Low power image of the whole trigeminal ganglia showing the ophthalmic V1 region of interest (box), scale bar = 200 μm. High power images of the ganglia from (C) an incision-control mouse, (D) CCI treated with vehicle, (E) CCI treated with MK8825, and (F) CCI treated with sumatriptan, scale bar = 100 μm.

Figure 4 Co-localization of CGRP and iNOS in the (A) trigeminal nucleus caudalis and (B) trigeminal ganglia after CCI injury. (A) Merged (yellow) CGRP (green) and iNOS (red) show similar distribution in the TN superficial lamina. (B) Merged CGRP and iNOS with DAPI (blue) nuclear stain shows majority of the ganglia cells are co-labeled.

Figure 5 ELISA quantification of CGRP levels in the caudal brainstem of CCI mice treated with vehicle, MK8825, and sumatriptan: CGRP was significantly reduced in the CCI sumatriptan-treated group compared to vehicle, \( **p < 0.01 \).

Conclusions

- Inhibition of CGRP release and binding using sumatriptan and MK8825, respectively, attenuated periorbital allodynic thresholds and levels of iNOS mRNA and protein in the trigeminal pathway.
- These findings, in combination with the co-localization of iNOS and CGRP, confirms that CGRP influences the production of iNOS.
- Blockade of CGRP reduces iNOS expression and is associated with improvement in headache-like behavior (periorbital allodynia).
- Findings indicate the interactions between CGRP and iNOS after head injury may contribute to the pathology underlying post-traumatic headache. Blockade of either CGRP and/or iNOS has therapeutic potential in the management of headache following head injury.