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# Research Submission

# An Exploratory Study of Salivary Calcitonin Gene-Related Peptide Levels Relative to Acute Interventions and Preventative Treatment With OnabotulinumtoxinA in Chronic Migraine

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**Objective.—To determine if baseline/interictal saliva calcitonin gene-related peptide (CGRP) levels would be lower in subjects with chronic migraine receiving onabotulinumtoxinA compared with those receiving saline.**

**Background.—CGRP is considered central to the pathogenesis of episodic migraine, but its relationship to chronic migraine is less understood. OnabotulinumtoxinA is an effective treatment for chronic migraine and has been demonstrated to inhibit the vesicular release of CGRP.**

**Methods.—This was an exploratory, randomized, placebo-controlled, crossover pilot study of 20 subjects that received onabotulinumtoxinA and saline injection (placebo). The amount of CGRP in saliva samples collected on a nonheadache or low headache day, and prior to and after treatment of a headache exacerbation was measured. Daily headache records, medications, and response to treatment were recorded in a diary.**

**Results.—A decrease in baseline/interictal saliva CGRP levels for subjects receiving onabotulinumtoxinA from 39.4 ± 7.5 pg CGRP/mg total protein after the first month to 25.5 ± 4.1 pg after the third month was observed. However, this difference did not reach significance nor was it significant when compared to the saline treatment. There was a reduction in the number of headache days for both onabotulinumtoxinA and saline over baseline throughout the active phases of the study. However, there was no statistical difference in headache days between groups. Subjects with a greater than 50% response to onabotulinumtoxinA had better 2-hour pain relief with acute treatment than non-responders to onabotulinumtoxinA or saline.**

**Conclusion.—While CGRP levels were not elevated during a migraine attack in chronic migraine subjects as has been reported in episodic migraine, there was an overall decrease in the baseline/interictal levels in response to onabotulinumtoxinA.**

**Key words: calcitonin gene-related peptide, chronic migraine, episodic migraine, pathophysiology, onabotulinumtoxinA, acute treatment**

**Abbreviations: CGRP calcitonin gene-related peptide, CM chronic migraine, EM episodic migraine**

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Calcitonin gene-related peptide (CGRP), a 37-amino acid peptide found in the peripheral, enteric, and central nervous system, is considered of central importance in the pathogenesis of acute attacks of migraine.[1,2] CGRP is a potent vasodilator and an important mediator of pain transmission.[3,4] Levels of CGRP are elevated during episodic migraine (EM) attacks and return to baseline with successful treatment or termination of the attack.[5] In a study of salivary CGRP levels during acute attacks of migraine treated with rizatriptan, it was observed that approximately two thirds of attacks demonstrated significant elevations of saliva CGRP during the premonitory or headache phase of a migraine attack.[6] In another EM study, saliva levels of CGRP were reported to be elevated over baseline in migraine attacks associated with autonomic nasal symptoms. Furthermore, there was a trend for baseline/interictal elevation of CGRP levels with increased attack frequency.[7]

CGRP, which is packaged in vesicles, is released following activation of trigeminal sensory neurons during a migraine attack.[8] While onabotulinumtoxinA is well recognized for its ability to block the vesicular release of acetylcholine from motor neurons,[9] results from studies have also demonstrated that the vesicular release of CGRP from sensory neurons is likewise blocked by onabotulinumtoxinA.[10,11] In both instances, the mechanism of transmitter release inhibition is through cleavage of the synaptosomal-associated protein 25 protein complex.[12] It seems reasonable to assume that when onabotulinumtoxinA is injected into the subcutaneous or muscle tissue, it is taken up both sensory and motor neurons. Both inhibitory effects are likely important in understanding the prophylactic benefit of onabotulinumtoxinA in chronic migraine (CM).[13]

Many drugs used to prevent or treat acute episodes of frequent EM are used to treat CM, but the only US Food and Drug Administration-approved preventative treatment for CM is onabotulinumtoxinA.[14] CM is a debilitating disease that is considered a complication of EM.[15] It affects 1-4% of adults in the general population,[16] but its lifetime occurrence in a medical population with EM is considerably higher and may approach 25%.[17] CM is the most common diagnosis in specialty headache clinics.[18] The natural history of CM is variable. In a population of CM patients followed over 2 years, 34% persisted in CM; 40% waxed and waned between CM and frequent EM; and 26% reverted to EM.[19]

Given the association of elevated CGRP levels in the pathogenesis of EM, we conducted a pilot study to determine temporal changes in saliva CGRP levels to better understand the relationship of CGRP in CM pathology and the response to preventive treatment with onabotulinumtoxinA, as well as acute rescue medications. We hypothesized that subjects treated with onabotulinumtoxinA would have lower salivary levels of CGRP when compared with subjects receiving only saline injections.

## METHODS

**Study Design.—**This study was conducted in accordance with the Declaration of Helsinki, all relevant

*Conflicts of Interest:* Dr. Roger Cady currently serves on several advisory boards: Allergan, Astellas, MAP Pharmaceuticals, Merck & Co, Inc., Novartis, Ortho-McNeil Neurologics, and Zogenix. He also receives research grants from Allergan, Boston Scientific, Bristol Myers, GlaxoSmithKline, Merck & Co, Inc., OptiNose, PuraMed Bioscience, and Zogenix. Dr. Cady provided consulting services for Allergan, Astellas, GlaxoSmithKline, Merck & Co., Inc., and Ortho-McNeil Neurologics.

Dr. Ira Turner has served as a consultant and/or speaker's bureau for Allergan, MAP, Nautilus, Zogenix and has received research support from Allergan.

Dr. Kent Dexter has nothing to disclose.

Ms. M.E. Beach has nothing to disclose.

Mr. Ryan Cady has nothing to disclose.

Dr. Paul Durham has received research support from Allergan.

Trial Registration: ClinicalTrials.gov NCT01071096

Study approved by Sterling IRB.

**Table 1.—Inclusion Criteria**

1) Generally healthy males or females between the ages of 18 and 65 years; able to provide informed consent; with a history of chronic migraine according to the criteria proposed as an appendix diagnosis in 2006 by the Headache Classification Committee.[21]
2) Subjects taking migraine preventive medications were on a stable dose for at least 6 weeks prior to screening and agreed not to start, stop, or change medication and/or dosage during the study period.
3) Females of childbearing potential agreed to urine pregnancy test at screening and a medically acceptable form of contraception. Medically acceptable forms of contraception include:
   a. Complete abstinence from intercourse from 2 weeks prior to administration of study drug throughout the study, and for a 5-day time interval after completion or premature discontinuation from the study to account for elimination of the study drug (a minimum of 7 days).
   b. Surgically sterile (hysterectomy or tubal ligation or otherwise incapable of pregnancy).
   c. Sterilization of male partner.
   d. Intrauterine device with published data showing lowest expected failure rate is less than 1% per year.
   e. Double barrier method (ie, 2 physical barriers or 1 physical barrier plus spermicide) for a least 1 month prior to visit 1 and throughout study.
   f. Acceptable hormonal contraceptives for at least 3 months prior to visit 1 and throughout study.

**Table 2.—Exclusion Criteria**

1) Females who were pregnant, planning to become pregnant during the study period, breast feeding, or are of childbearing potential and not practicing a reliable form of birth control (see inclusion criteria).
2) Have headache disorders outside IHS-defined chronic migraine.
3) Evidence of underlying pathology contributing to their headaches.
4) Pathology of the salivary glands such as sialadenitis (eg, Sjorgen's syndrome, viral or bacterial sialadenitis) or condition or symptom that would alter the content of saliva.
5) Any medical condition that may increase their risk with exposure to Botox including diagnosed myasthenia gravis, Eaton–Lambert syndrome, amyotrophic lateral sclerosis, or any other significant disease that might interfere with neuromuscular function.
6) Profound atrophy or weakness of muscles in the target areas of injection.
7) Skin conditions or infections at any of the injection sites.
8) Allergy or sensitivities to any component of test medications.
9) Active major psychiatric or depressive disorders including alcohol/drug abuse.
10) Meets International Headache Society criteria for Medication Overuse with opioid or butalbital containing products.
11) Is planning or requiring surgery during the study.
12) A history of poor compliance with medical treatment as determined by the investigator.
13) Is currently participating in an investigational drug study or has participated in an investigational drug study within the previous 30 days of the screening visit.

US federal regulations, and in compliance with the International Conference on Harmonization Guideline for Good Clinical Practice. The study protocol, informed consent, and all appropriate study-related documents were approved by an independent Institutional Review Board/Ethics Committee. Written informed consent was obtained from each patient prior to any protocol-related activities. The study was registered at ClinicalTrials.gov (NCT01071096).

This was an exploratory, randomized, placebo-controlled, crossover pilot study conducted at 2 private clinics: the Headache Care Center in Springfield, MO and Island Neurological Associates in Plainview, NY. The baseline was determined at screening by collecting headache history retrospectively at visit 1 and used to define the frequency of headache days and document the diagnosis of CM. Twenty subjects meeting inclusion and exclusion criteria (Tables 1 and 2) were randomized 1:1 to initially receive either onabotulinumtoxinA (group A) or saline injection (group B). Randomization of subjects was performed by a supervisory individual not associated with the study, who numbered study medications in a manner which was blinded to subject, coordinator, and investigator. The randomization scheme was generated using the web site: (http://www.randomization.com).

At their first visit, subjects received either a dose of 155 IU onabotulinumtoxinA purified neurotoxin complex (0.1 cc per injection site) or equivalent volume of saline in 31 fixed sites in the head and neck as previously described in the PREEMPT Study.[20] Optional additional dosing in the occipitalis, temporalis, and trapezius was allowed at the discretion of the investigator. The decision on how many additional units were injected took into account the following criteria: patient-reported usual location of predominant pain; severity of the muscle tenderness while palpating the muscle prior to injection; and

clinician's best judgment on the potential benefit of additional doses in the specified muscles (eg, large muscle size). Four months after injection of onabotulinumtoxinA or saline, the groups were crossed over and injected in 31 fixed sites with either onabotulinumtoxinA or saline. At visit 1, group A subjects received onabotulinumtoxinA, while at visit 5 were injected with saline and monitored for an additional 3 months. Group B subjects were initially injected with saline at visit 1, then at visit 5 received onabotulinumtoxinA. Subjects were blinded to the randomized drug schedule assignment, but were informed that they would be receiving both placebo and onabotulinumtoxinA during the study.

Treatment response to onabotulinumtoxinA and acute medication was monitored via daily paper diary records by documenting migraine headache days, duration of each migraine, migraine severity, and associated symptoms. During the study, subjects treated acute exacerbations of migraine using their usual medications, approved by the investigator at the randomization visit. They also recorded medications and dosages used for acute treatment on the daily headache diary. Migraine preventive medication and routine concomitant medications considered necessary for the subject's welfare were allowed when maintained at a stable dose and regimen for the duration of the study period and at the discretion of the investigator.

Saliva samples were collected at monthly intervals and analyzed for CGRP. Subjects were instructed to collect saliva for analysis at the following 3 times each month: baseline/interictal level when a subject was without headache or at their lowest level of chronic persistent headache; onset of a headache pain that was at least 1 point worse on a scale of 0-3 than baseline level and prior to treatment with acute therapy; and 2 hours following usual acute treatment of headache as noted above.

Subjects returned monthly throughout the 7-month study. At each visit, saliva samples were returned, headache diary was reviewed, vital signs were completed, and a urine pregnancy test collected from any subject of childbearing potential. Medical records were updated and adverse events were noted.

**Saliva Collection and CGRP Analysis.—**Saliva collection and analysis was performed as previously described with the following modifications.[20] Subjects were instructed to gently chew a piece of citric flavored gum for 5 minutes. During the first 2 minutes, saliva was discarded to prevent the mixing of unstimulated and stimulated saliva. While continuing to gently chew the gum, saliva was expectorated into a chilled 15 mL conical tube until 5 mL of saliva was collected, and the tube immediately placed in a freezer. Saliva samples were analyzed using a commercially available CGRP RIA, following manufactures protocol (Bachem, Torrance, CA, USA) and values normalized to total volume and total amount of protein as determined by the Bradford method.

**Statistical Analysis.—**This was a pilot study in which no a priori analysis was conducted. The primary outcome measure was a reduction in saliva CGRP levels in CM subjects receiving onabotulinumtoxinA compared with saline. Secondary outcome measures included determining if there was a reduction in the number of headache days in response to onabotulinumtoxinA and evaluating whether there was a reduction in the 2-hour pain scores in onabotulinumtoxinA responders. Subgroup analysis between responders and nonresponders was conducted which is not as reliable as a priori defined analysis. Data were analyzed by 2-way repeated measures ANOVA, followed by Tukey or Mann–Whitney *U*-tests for post-hoc analysis using IBM SPSS v20 (Armonk, NY, USA). Statistical significance was considered at $P < .05$.

## RESULTS

Twenty subjects, male (5) and female (15), with at least a 3-month history of stable CM, as defined by the Appendix definition for CM proposed by Olesen et al[21] were screened for this study. Recruitment began on July 8, 2010 and ended on May 10, 2011 with the enrollment of the twentieth subject. All subjects were Caucasian with an average age of 48.5 (standard deviation = 12.87). Only 1 female subject did not complete the study, as she was lost to follow-up.

**CGRP Analyses.—**The mean CGRP level for all subjects at the start of the study during a headache free period was $32 \pm 3$ pmol/mg total protein.

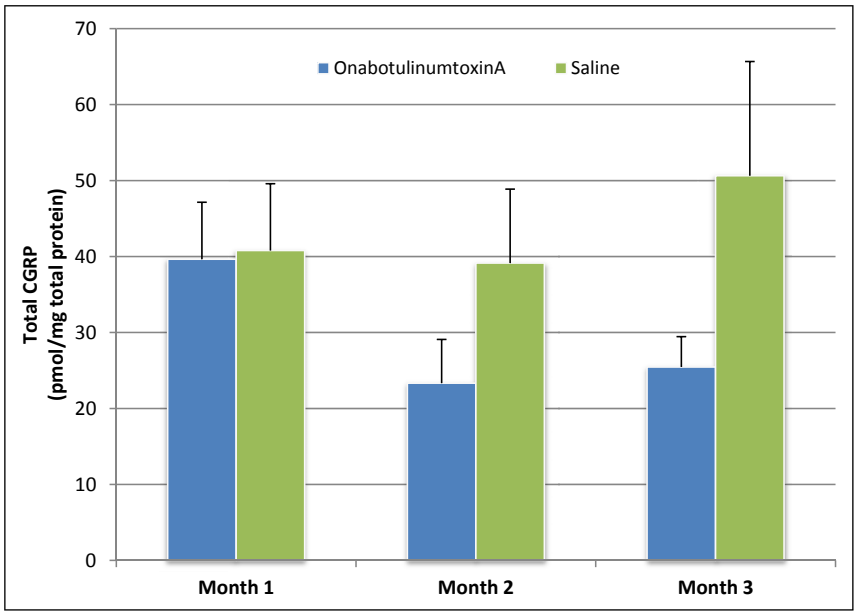


**Fig 1.—Monthly change in saliva calcitonin gene-related peptide (CGRP) levels in response to onabotulinumtoxinA or saline.**

CGRP levels did not decrease from initial levels in the first month following injection in subjects receiving onabotulinumtoxinA or saline. At months 2 and 3 following injections, there was a decrease in baseline/interictal CGRP levels for group A (onabotulinumtoxinA), but not for group B (saline) (Fig. 1). The difference at the 3-month time period narrowly missed statistical significance, which is likely due to the small number of subjects ($n = 19$).

**Attack Associated Changes in Saliva CGRP.—** There were no significant differences in saliva CGRP levels at the initiation of attack or 2-hours post treatment when compared with baseline/interictal levels for any treatment group (Fig. 2). Specifically, there was no increase of saliva CGRP levels associated with onset or increase in headache severity, nor was there a decrease in saliva CGRP associated with acute treatment. This was true regardless

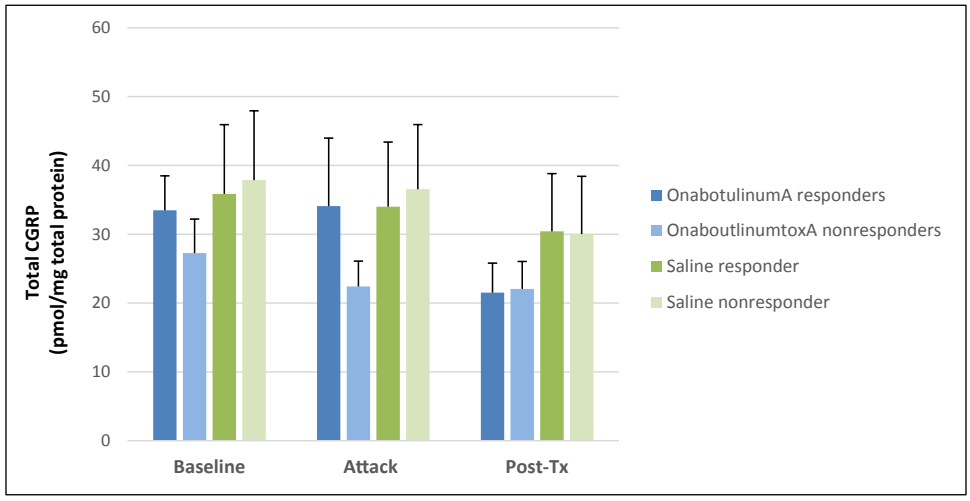


**Fig 2.—Changes in saliva calcitonin gene-related peptide (CGRP) levels during an attack and following treatment. The level of CGRP in saliva is reported in pmol/mg total protein for subjects injected with onabotulinumtoxinA (blue) or saline (green) that experienced a ≥50% decrease in number of headache days per month from their initial baseline levels (responders).**

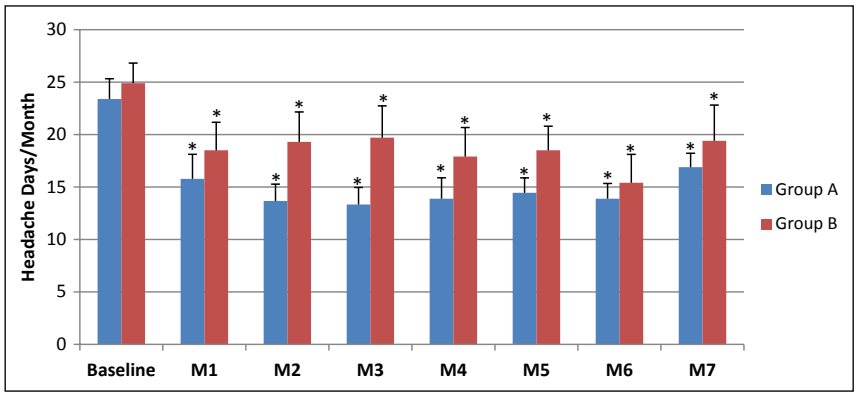


**Fig 3.—Average number of migraine headache days per month. The average number of headache days is reported at the initiation of the study (baseline) and at monthly (M) intervals following treatment. Group A received injections of onabotulinumtoxinA initially and then saline at the beginning of month 5 while group B received injections of saline initially and then onabotulinumtoxinA at the beginning of month 5. *$P \leq .05$ from baseline.**

of group assignment. Furthermore, no difference was observed between subjects that responded to either onabotulinumtoxinA or saline. A responder was defined as a subject who experienced a ≥50% decrease in number of headache days per month from their initial baseline levels.

**Changes in Migraine Headache Days.—**Injections of either onabotulinumtoxinA or saline resulted in a significant reduction of headache days from baseline, although the change from baseline at each month was greater for those subjects receiving onabotulinumtoxinA (Fig. 3). In subjects initially injected with onabotulinumtoxinA, but not saline, there was a reduction in the number of headaches per month below the threshold used to define CM during months 2-6. However, there was no statistically significant difference in headache day reduction between treatment groups at any time during the study. This is not unexpected given a population size of 19 subjects. This reduction in migraine headache days has been documented in other studies of onabotulinumtoxinA.[22] OnabotulinumtoxinA and saline injections are well tolerated in subjects with CM. There were no serious adverse events reported in this study and no differences in the adverse event profile between onabotulinumtoxinA and saline. These results are consistent with other studies of onabotulinumtoxinA except for the lower incidence of neck pain.[23] The authors believe that this relates to injector experience.

**Acute Treatment.—**Pain levels prior to acute treatment were similar for all groups (Fig. 4). The response to acute treatment at 2-hours postdose was statistically superior for those responding (≥50% reduction in headache days) to onabotulinumtoxinA compared with saline nonresponders ($P = .04$). OnabotulinumtoxinA responders exhibited a better response to acute treatment over nonresponders although the average pain levels were not statistically different (Fig. 4). Interestingly, saline responders and responders to onabotulinumtoxinA had similar positive responses at 2-hours post-acute treatment.

**Pre- and 2-Hour Post-Pain Scores for Specific Abortive Medications.—**A reduction in average pain level was reported for each of the abortive treatments (Fig. 5). We had hypothesized acute medications blocking CGRP release, such as triptans, may be more efficacious as an abortive treatment than other medications. However, medications such as opioids, nonsteroidal anti-inflammatory drugs, and ergotamine caused a similar level of pain reduction. The different therapeutic response with acute treatment based on the various classes of drugs is of interest, but should be interpreted cautiously because of the small number of subjects in each group (Fig. 5). The data did not support any differences in response to different classes of acute medications.

## DISCUSSION

This was a small pilot study, and the results need to be interpreted cautiously. However, there are several observations in this study worthy of discussion and further research. It is essential to emphasize

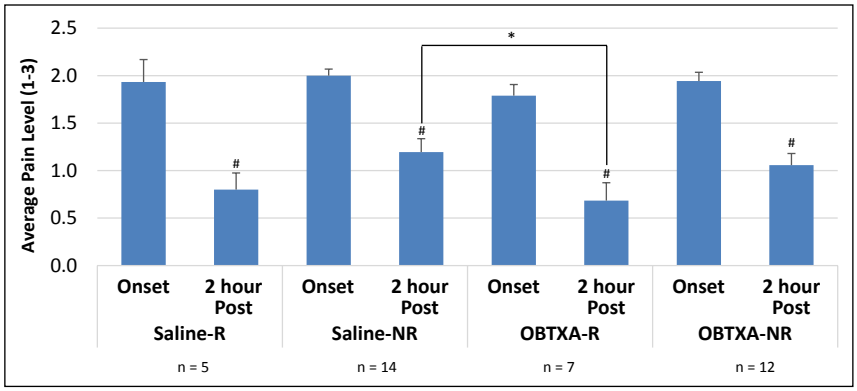


**Fig 4.—Pre- and 2-hour post-treatment pain scores. The average pain level at onset of an attack or 2 hours post-treatment with a rescue medication is reported for subjects that experienced a ≥50% reduction in the number of headache days/month in response to either onabotulinumtoxinA (OBTXA) or saline. R, responders; NR, nonresponders. *$P$ = .04 for ON-R vs saline-NR; #$P$ > .05 from onset to 2 Hr. Post.**

that the focus of the study was to gain insight into the relationship of salivary CGRP levels and CM pathophysiology. This was not an efficacy study, given the small number of enrolled subjects. Despite its limitations, this study did produce some intriguing results.

In 2004, the International Classification of Headache Disorders for the first time divided migraine into 2 distinct entities: episodic and CM. It is generally presumed that these 2 forms of migraine are related pathophysiologically,[24,25] but this assumption remains unproven. In EM, there is evidence that peripheral activation of the trigeminovascular system precedes central activation,[26] and as central sensitization progresses to the point of cutaneous allodynia, acute intervention with triptans is less likely to be successful.[27] Burstein et al have suggested that a throbbing headache is the hallmark of peripheral sensitization, and cutaneous allodynia is the clinical marker of central sensitization.[28] Subjects with CM frequently report throbbing headaches and frequently have

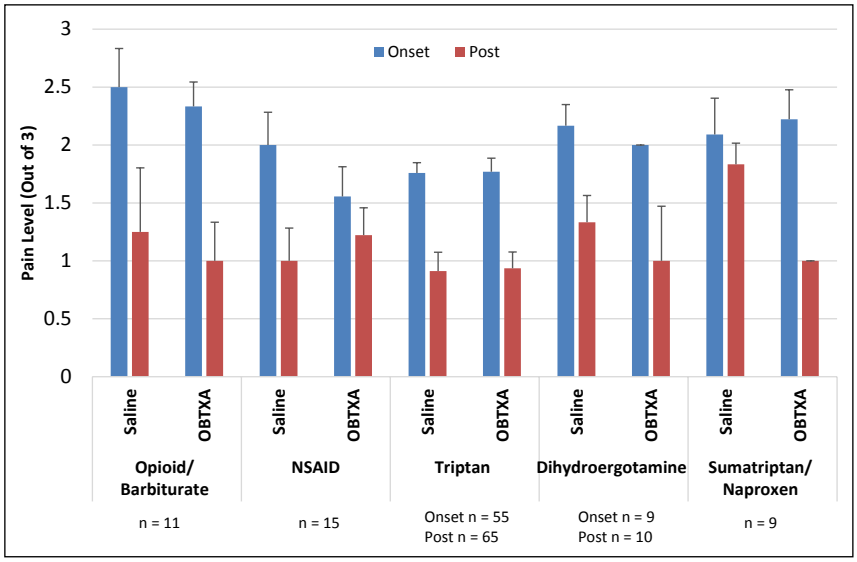


**Fig 5.—Pre- and 2-hour post-headache scores for specific abortive medications. The average pain level at onset of an attack or 2 hours post-treatment with a rescue medication is reported for subjects in response to either onabotulinumtoxinA (OBTXA) or saline.**

cutaneous allodynia even between episodes of more severe headache.[29] This suggests that in CM, the headache and migraine symptoms are driven by both peripheral and central mechanisms.

The role of CGRP in EM has been acknowledged for decades. CGRP released from trigeminal afferents during migraine results in vasodilation and initiation of pain signaling from the periphery.[30] Data from this study suggest onabotulinumtoxinA reduces salivary levels of CGRP that are regulated by peripheral nerves. The reduction in CGRP, secondary to onabotulinumtoxinA, may be a component of its mechanism of action as a prophylactic in CM. Of further interest, an elevation of saliva CGRP that has been associated with exacerbations of migraine headache in EM was not demonstrated in this study. Nor was there a reduction in CGRP after acute treatment with a triptan or any other acute treatment. These findings are suggestive that the threshold for central activation from peripheral input is significantly less in CM than EM and support the notion that central mechanisms are a prominent component of the pathophysiology of CM.

An unexpected finding in this exploratory study was that subjects responding to onabotulinumtoxinA with a ≥50% reduction in headache days had a statistically superior response to acute intervention vs saline nonresponders and narrowly missed statistical significance when comparing nonresponders with onabotulinumtoxinA. One of the hypotheses at the start of the study was that responders to onabotulinumtoxinA would be more responsive to triptans. However, we observed a similar reduction in average pain score regardless of the class of rescue medication used to abort a migraine attack. These data may challenge the arbitrary restrictions placed on specific medications by the headache community for use in CM because all classes of acute medication appear to have a near equal degree of efficacy. Ideally, this study will be a catalyst for a larger clinical trial assessing use of different classes of acute medications in treatment of CM.

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