Original Article

Cephalalgia
An International Journal of Headache

International Headache Society

Cephalalgia
2020, Vol. 40(9) 892–902


Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/0333102420938652
journals.sagepub.com/home/cep
SAGE

Ubrogepant does not induce latent sensitization in a preclinical model of medication overuse headache

Edita Navratilova[1], Sasan Behravesh[2], Janice Oyarzo[2], David W Dodick[3], Pradeep Banerjee[4] and Frank Porreca[1,2]

Abstract

Background: Ubrogepant, a small-molecule calcitonin gene-related peptide receptor antagonist, was recently approved as an oral medication for the acute treatment of migraine. This study aimed to determine whether ubrogepant shows efficacy in a preclinical model of migraine-like pain and whether repeated oral administration of ubrogepant induces latent sensitization relevant to medication overuse headache in rats.

Methods: A "two-hit" priming model of medication overuse headache was used. Female Sprague-Dawley rats received six oral doses of sumatriptan 10 mg/kg over 2 weeks to induce latent sensitization (i.e. "priming"). Cutaneous allodynia was measured periodically over 20 days in the periorbital and hindpaw regions using von Frey filaments. The rats were then subjected to a 1-hour bright light stress challenge on two consecutive days. At the start of the second bright light stress exposure, oral sumatriptan 10 mg/kg, oral ubrogepant 25, 50, or 100 mg/kg, or vehicle was administered; thereafter, cephalic and hindpaw sensory thresholds were monitored hourly over 5 hours to determine the efficacy of ubrogepant in reversing bright light stress-induced cutaneous allodynia. A dose of ubrogepant effective in the medication overuse headache model (100 mg/kg) was then selected to determine if repeated administration would produce latent sensitization. Rats were administered six oral doses of ubrogepant 100 mg/kg, sumatriptan 10 mg/kg (positive control), or vehicle over 2 weeks, and cutaneous allodynia was evaluated regularly. Testing continued until mechanosensitivity returned to baseline levels. Rats were then challenged with bright light stress on days 20 and 21, and periorbital and hindpaw cutaneous allodynia was measured. On days 28 to 32, the same groups received a nitric oxide donor (sodium nitroprusside 3 mg/kg, i.p.), and cutaneous allodynia was assessed hourly over 5 hours.

Results: Sumatriptan elicited cutaneous allodynia in both cephalic and hindpaw regions; cutaneous allodynia resolved to baseline levels after cessation of drug administration (14 days). Sumatriptan priming resulted in generalized and delayed cutaneous allodynia, evoked by either bright light stress (day 21) or nitric oxide donor (day 28). Ubrogepant dose-dependently blocked both stress- and nitric oxide donor-induced cephalic and hindpaw allodynia in the sumatriptan-induced medication overuse headache model with a 50% effective dose of ~50 mg/kg. Unlike sumatriptan, ubrogepant 100 mg/kg in repeated effective doses did not produce cutaneous allodynia or latent sensitization.

Conclusions: Both ubrogepant and sumatriptan demonstrated efficacy as acute medications for stress- and nitric oxide donor-evoked cephalic allodynia in a preclinical model of medication overuse headache, consistent with their clinical efficacy in the acute treatment of migraine. However, in contrast to sumatriptan, repeated treatment with ubrogepant did not induce cutaneous allodynia or latent sensitization. These studies suggest ubrogepant may offer an effective acute treatment of migraine without risk of medication overuse headache.

Trial Registration Number: Not applicable

[1]Department of Pharmacology, Arizona Health Sciences Center, University of Arizona, Tucson, AZ, USA
[2]Department of Collaborative Research, Mayo Clinic, Scottsdale, AZ, USA
[3]Department of Neurology, Mayo Clinic, Scottsdale, AZ, USA
[4]Allergan plc, Madison, NJ, USA

Corresponding author:
Frank Porreca, Department of Pharmacology, University of Arizona, Tucson, AZ 85724, USA.
Email: frankp@medadmin.arizona.edu

Keywords
Medication overuse headache, allodynia, ubrogepant, sumatriptan, migraine

Date received: 8 April 2020; revised: 21 April 2020; 4 May 2020; accepted: 5 May 2020

Introduction

Effective management of patients' migraine is often inadequate, and treatments are associated with unintended consequences. Frequent use of acute medications, including opiates, cannabinoids, barbiturates, and nonsteroidal anti-inflammatory drugs (NSAIDs), as well as migraine-specific medications (e.g. triptans), can lead to medication overuse headache (MOH), characterized by increased frequency of headache attacks, increased pain intensity, and increased disability (1–3). Novel drugs with new mechanisms of action have recently been approved, but it is unknown whether these will also carry the risk of inducing MOH in vulnerable patients. MOH is defined in the *International Classification of Headache Disorders* (ICHD-3) as a headache that (a) develops as a consequence of regular overuse of symptomatic headache medication in a person with a preexisting headache disorder and (b) occurs on >14 days/month for >3 months and cannot be better accounted for by another ICHD-3 diagnosis (4,5). The prevalence of MOH has been estimated at 1% to 2% in the general population and is among the top 20 causes of years lived with disability (6–11). Multiple drug classes are used for the acute treatment of headaches and, to date, almost all are implicated in the development of MOH (1,2,12,13). Medication overuse is a risk factor in the transformation of episodic migraine to chronic migraine, which is associated with decreased quality of life (7,14–16). The need for novel drugs that are effective in the acute treatment of migraine but that do not provoke development of MOH is desired by both patients and clinicians.

We have used a preclinical model of MOH and reported that a period of treatment with acute medications, including sumatriptan, naratriptan, cannabinoids, and morphine, given by subcutaneous osmotic minipump or by repeated systemic injections, can result in a long-lasting increase in sensitivity to provocative stimuli that are thought to be associated with induction of migraine in humans; these stimuli include stress and nitric oxide (NO) donors. Such "two-hit" priming models reveal a "latent sensitization" state that may be relevant to MOH (17–19). The outcomes of challenges with provocative stimuli in previously primed animals include delayed and generalized cutaneous allodynia (CA) measured in cephalic and extracephalic regions (20). This CA emerges gradually after exposure to a stimulus, peaking generally at 2–3 hours poststimulus and subsiding within 5 hours, and is accompanied by increased levels of calcitonin gene-related peptide (CGRP) measured in jugular vein blood (21). Because thermal and mechanical cutaneous hypersensitivity are often seen in patients with migraine (22–24) and MOH (25), the occurrence of CA, particularly facial or periorbital CA, is often used as a surrogate for headache in animal models (24,26–28). In patients, CA usually occurs ictally but often persists interictally (24) and has higher prevalence in patients with chronic migraine, compared with episodic migraine. Interestingly, medications that induce MOH are nevertheless effective in acute treatment of migraine attacks in humans (2,12). Stress- or NO donor-induced allodynia in animals with latent sensitization can be blocked by acute medications, including sumatriptan, morphine, and NSAIDs, or by pretreatment with a CGRP antibody (28–30). Collectively, these observations suggest that, in the absence of injury, repeated exposure to acute medications may produce a state of increased vulnerability to migraine triggers through a CGRP-dependent mechanism.

The primary aims in this preclinical study were to determine whether ubrogepant, a small-molecule CGRP receptor antagonist (31), is effective in reversing allodynia in rats primed with sumatriptan and whether repeated administration of doses effective in the preclinical model of MOH produces latent sensitization similar to that of sumatriptan, a 5-hydroxytryptamine 1B/1D receptor agonist (18).

Methods

Animals

Adult female Sprague-Dawley (SD) rats (Harlan Laboratories Inc., Indianapolis, IN, USA) initially weighing 135 to 150 grams were maintained three per cage in a climate-controlled room at $22 \pm 2^{\circ}C$ on a 14-hour light/10-hour dark cycle (lights on at 5 am, off at 7 pm) with free access to food and water. Studies were conducted during the animals' light cycle following approval by the Institutional Animal

Care and Use Committee of Mayo Clinic Arizona. Sixty rats were used in these studies. Animals were randomly divided into treatment groups; blinding was not possible in these experiments because of staff unavailability. Group size requirements n = 4–7 rats/group were determined from previous experiments and predicted effect sizes of 3 for hindpaw and 2.5 for periorbital allodynia using G*Power 3.1 with significance $\alpha = 0.05$ and statistical power $(1\text{-}\beta) = 0.9$.

Drugs

Ubrogepant provided by Allergan (Madison, NJ, USA) was dissolved in polyethylene glycol (PEG); sumatriptan succinate obtained from Abmole Bioscience (Houston, TX, USA) was dissolved in sterile water.

Dosing

Six doses of sumatriptan were administered orally by gavage at 10 mg/kg over 2 weeks to establish the MOH model. Ubrogepant 25, 50, or 100 mg/kg or sumatriptan 10 mg/kg was given orally by gavage as an acute treatment to sumatriptan-primed rats at the time of stress or NO donor induction. In the repeated ubrogepant dosing experiment, six doses of ubrogepant 100 mg/kg were administered by gavage.

Pain measurement: Behavioral assessment of cutaneous allodynia

Each rat was placed in a plexiglass chamber with mesh flooring in an isolated and quiet room with no foot traffic and acclimated for 2 hours/day for 3 days. Mechanical periorbital and hindpaw allodynia was measured using calibrated von Frey monofilaments prior to the priming drug treatments (baseline) and during a 20-day period after priming. Prior to testing, each rat was allowed 2 hours to acclimate. In the von Frey tests, the mechanical stimulation was incrementally increased until a positive response was obtained, and then was decreased until a negative result was observed. This was repeated until three changes in behavior were determined ("up and down" method) (18). The 50% paw withdrawal threshold was determined as $(10[Xf+k\delta])/10{,}000$, where Xf = value of last von Frey filament used, k = Dixon value for positive/negative pattern, and δ = logarithmic difference between stimuli. Cutoff values were 8 g (4.93 filament) for the periorbital region and 15 g (5.18 filament) for the hindpaw. The filaments were applied with an even pressure that ensured the filament arched slightly at the periorbital area or in the hindpaw region. Swiping of the face, shaking of the head, and/or turning away from the filament were registered as positives for periorbital assessment. Moving away from the stimulus or rearing up was disregarded. For hindpaw assessment, swift withdrawal, shaking, or licking of the paw was deemed a positive response; moving away was not. All behavioral testing was performed by the same person (JO).

Migraine triggers

Bright light stress challenge: Unrestrained rats were placed individually in plexiglass cages and exposed to bright light (1000 lumens) from two LED lights placed on opposite sides of the cage for 1 hour each on two consecutive days (usually days 20 and 21) when mechanical thresholds were back at basal levels (17). On the second day of bright light stress (BLS), mechanical periorbital and hindpaw allodynia was measured hourly over a 5-hour period after BLS.

Nitric oxide donor: After resolution of MOH-induced allodynia, rats were administered an NO donor (sodium nitroprusside 3 mg/kg, i.p.); mechanical facial and hindpaw allodynia was measured over a 5-hour period after NO donor administration.

Experimental design overview

Experiment 1: Establishment of the sumatriptan MOH model and evaluation of the anti-allodynic efficacy of sumatriptan in stress-induced allodynia.

Twelve female SD rats were administered six oral doses of sumatriptan 10 mg/kg in water on days 0, 2, 6, 8, 9, and 11 to establish the MOH model. Tactile (periorbital and hindpaw) withdrawal responses before (baseline) and periodically over 20 days after sumatriptan treatment were assessed to measure allodynia. The rats were exposed to BLS for 1 hour on day 20. On day 21, the rats were divided into two groups (n = 6/group): Group 1 received water orally, and group 2 received sumatriptan 10 mg/kg orally. Immediately after treatment, the rats were exposed to BLS for 1 hour; periorbital and hindpaw allodynia was assessed at 1-hour time points over 5 hours. On day 31, the same rats were again exposed to BLS for 1 hour. The next day (day 32), they were treated with either water or sumatriptan as before; they were immediately exposed to BLS for 1 hour, and CA was assessed.

Experiment 2: Determination of ubrogepant efficacy in reversal of BLS- and NO donor-related allodynia in sumatriptan-primed rats.

Twenty-four female SD rats were administered six oral doses of sumatriptan 10 mg/kg in water on days 0, 3, 5, 7, 10, and 12 to establish the MOH model. Tactile withdrawal responses were measured before (baseline) and periodically over 20 days to assess CA. On day 20, the rats were exposed to stress (bright lights for 1 hour).

On day 21, prior to BLS exposure, they were divided into four groups (n = 6/group) based on ubrogepant treatment doses: Group 1 received PEG (vehicle for ubrogepant); group 2, ubrogepant 25 mg/kg; group 3, ubrogepant 50 mg/kg; and group 4, ubrogepant 100 mg/kg. Immediately, all rats were exposed to BLS for 1 hour; periorbital and hindpaw allodynia was assessed at 1-hour time points over 5 hours. On day 28, rats received PEG, or ubrogepant 25, 50, or 100 mg/kg, followed immediately by NO donor (sodium nitroprusside 3 mg/kg, i.p.); CA was assessed over a 5-hour time course after NO donor administration.

Experiment 3: Determination of the consequences of ubrogepant priming.

Twenty-four female SD rats were divided into three groups based on drug treatment on days 0, 2, 4, 8, 9, and 12: Group 1, PEG (vehicle for ubrogepant), n = 6; Group 2, sumatriptan 10 mg/kg, n = 6; Group 3, ubrogepant 100 mg/kg, n = 12. CA was assessed at baseline and periodically over 20 days from the first day of drug treatment. Rats were then exposed to BLS for 1 hour on 2 consecutive days (days 20 and 21), and periorbital and hindpaw allodynia was assessed hourly over 5 hours. Acute treatment was not given prior to the two BLS exposures. The same animals were exposed to an NO donor (sodium nitroprusside 3 mg/kg, i.p.) on day 30, and CA was measured.

Data analysis

GraphPad Prism 8 (GraphPad Software, La Jolla, CA) was used for statistical data analysis. For one experimental group, one-way repeated-measures analysis of variance (ANOVA) with Dunnett post hoc test was used to determine significant differences from the baseline. Two-way repeated-measures ANOVA with time as a within-subject factor and treatment as a between-subjects factor was used to compare multiple treatment groups. The effect of time following priming with BLS or NO donor was compared to the baseline with Dunnett post hoc multiple-comparisons test. The Tukey post hoc test was used to analyze significant differences between treatment groups. Effectiveness of drug treatment was estimated from the area under the curve (AUC) of the withdrawal threshold time course. AUC was calculated for each rat as an area of negative peaks below the 8 g (periorbital) or 15 g (hindpaw) cutoff thresholds between the 1- and 5-hour time points. Percent effect was calculated using the formula 100*[AUC(0)-AUC(X)]/AUC(0), where AUC(0) = average AUC in vehicle-treated group and AUC (X) = AUC of animals that received treatment X. Dose-response curves for ubrogepant were generated by plotting the percentage of effects as a function of $\log_{10}$(dose), and a linear regression analysis was performed to calculate the 50% effective dose values. Statistical significance was set at $p < 0.05$. All data are presented as mean ± standard error of the mean.

Results

Repeated administration of sumatriptan elicits generalized cutaneous allodynia and promotes latent sensitization (MOH model)

Cephalic and hindpaw allodynia was observed during the period of sumatriptan dosing but resolved by day 20 (Experiment 1, Figures 1(a) and (b)). One-way repeated-measures ANOVA confirmed significantly reduced withdrawal thresholds in both periorbital and hindpaw regions (see Table 1). Exposure to BLS on two consecutive days (days 20 and 21) produced significant CA in the sumatriptan-primed rats that received water on day 21; this CA was generalized and delayed, peaking at ~1 hour and persisting for ~5 hours (Figures 1(c) and (d); see Table 1 for statistical results). This state of latent sensitization was long-lasting because exposing the same rats to BLS on days 31 and 32 again produced significant periorbital and hindpaw CA (Figures 1(e) and (f), Table 1).

Acute administration of sumatriptan is effective in blocking stress-induced allodynia in MOH rats

Compared with MOH rats that received vehicle treatment, those that received an oral dose of sumatriptan 10 mg/kg just prior to the second BLS exposure exhibited significantly reduced CA (Figures 1(c) and (d), Table 1). Furthermore, when the same rats were exposed to BLS on days 31 and 32, sumatriptan pretreatment on day 32 was still effective in inhibiting CA (Figures 1(e) and (f), Table 1). The efficacy of sumatriptan in reversing CA was estimated to be 44% and 42%, respectively, from the periorbital and hindpaw CA measurements on day 21 and 64% and 63%, respectively, from the periorbital and hindpaw CA measurements on day 32.

Ubrogepant dose-dependently reverses bright light stress- and nitric oxide donor-induced allodynia in sumatriptan-primed rats

A separate group of rats was treated repeatedly with six oral doses of sumatriptan to induce the MOH model (Experiment 2, Figures 2(a) and (b), Table 1). Following resolution of CA, the efficacy of 25, 50, and 100 mg/kg doses of ubrogepant in reversing BLS-induced allodynia was assessed and compared with the efficacy of vehicle. Ubrogepant administered on day 21 prior to the second day of BLS reversed allodynia in a

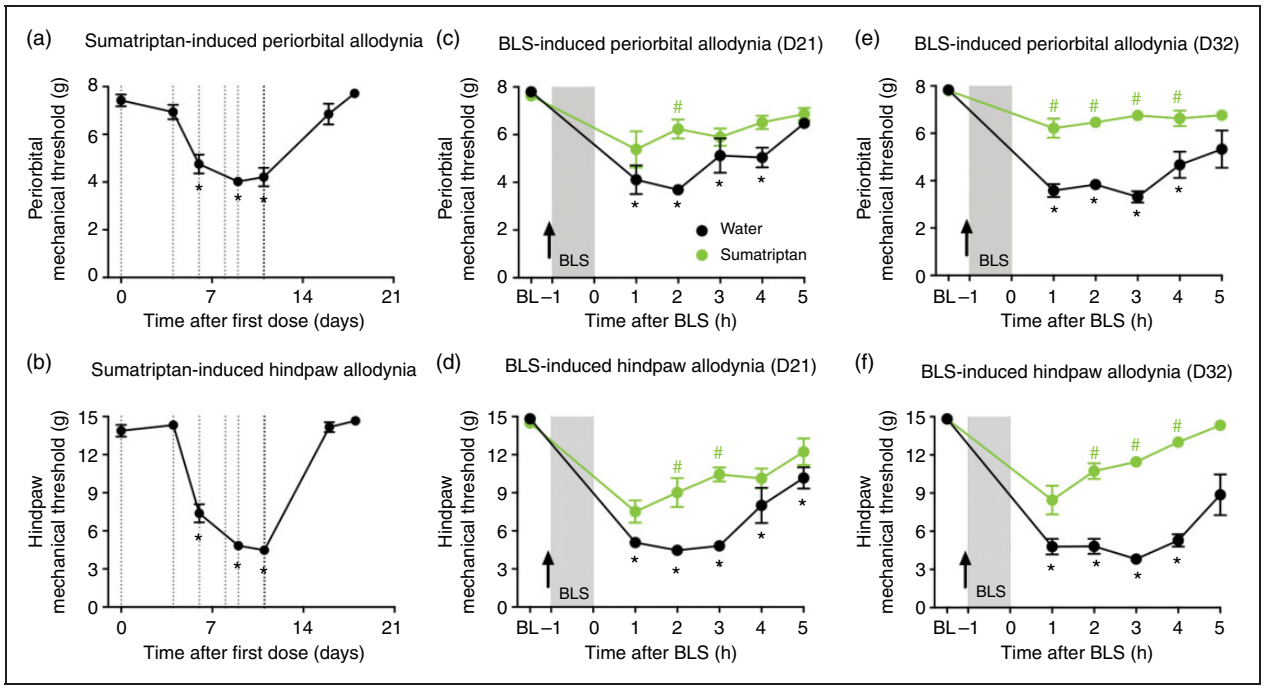

Figure 1. Female Sprague-Dawley rats received six oral doses of sumatriptan 10 mg/kg in water on days 0, 2, 6, 8, 9, and 11 (administration days indicated by dotted lines) to establish the model of medication overuse headache. Periorbital (a) and hindpaw (b) allodynia was measured over 20 days. After termination of sumatriptan dosing and resolution of sumatriptan-induced allodynia, rats were exposed to bright light stress (BLS) on two consecutive days (days 20 and 21); periorbital (c) and hindpaw (d) allodynia was assessed hourly over 5 hours on the second day of BLS. Compared with water, sumatriptan administered immediately prior to BLS on day 21 significantly blocked stress-induced allodynia. The same rats were again exposed to BLS on days 31 and 32; periorbital (e) and hindpaw (f) allodynia was assessed on day 32. Sumatriptan administered immediately prior to BLS on day 32 significantly blocked stress-induced allodynia. Data are mean ± standard error of the mean; n = 6 rats/group.
*Significantly reduced withdrawal thresholds from the baseline.
#Significant difference between sumatriptan and water-treated rats.

dose-dependent manner (Figures 2(c) and (d), Table 1). On day 28, administration of the NO donor sodium nitroprusside to the same groups of MOH rats elicited allodynia in the PEG-pretreated animals, but ubrogepant dose-dependently blocked NO donor-induced allodynia (Figures 2(e) and (f), Table 1). Ubrogepant dose-response curves for the reversal of stress- and NO donor-induced periorbital and hindpaw CA demonstrate an approximately linear relationship within the selected dose range (Figures 2(g) and (h)). In linear regression analysis, 50% effective dose values were estimated to be 50 and 52 mg/kg, respectively, for BLS-induced periorbital and hindpaw allodynia and 65 and 70 mg/kg, respectively, for NO donor-induced periorbital and hindpaw allodynia. The efficacy of ubrogepant 100 mg/kg in reversing CA was estimated to be 76% and 77%, respectively, from the periorbital and hindpaw CA measurements on day 21 (BLS-induced CA) and 67% and 70%, respectively, from the periorbital and hindpaw CA measurements on day 28 (NO donor-induced CA).

Repeated treatment with a dose of ubrogepant that is effective in the MOH model does not produce cutaneous allodynia and does not promote latent sensitization

The 100 mg/kg dose of ubrogepant was selected from the ubrogepant dose-response curves and was administered six times over 14 days to investigate whether repeated ubrogepant exposure induces MOH and latent sensitization in a way similar to sumatriptan exposure. The 100 mg/kg dose of ubrogepant is approximately twice the estimated acute anti-allodynic 50% effective dose and is equivalent to, or more effective than, the 10 mg/kg dose of sumatriptan. Unlike repeated administration of sumatriptan, repeated administration of ubrogepant 100 mg/kg had no effect on CA during the 14-day dosing regimen; PEG, the vehicle for ubrogepant, also had no effect (Experiment 3, Figures 3(a) and (b), Table 1). Exposure of sumatriptan-primed animals to BLS on 2 days (days 20 and 21) elicited significant periorbital and

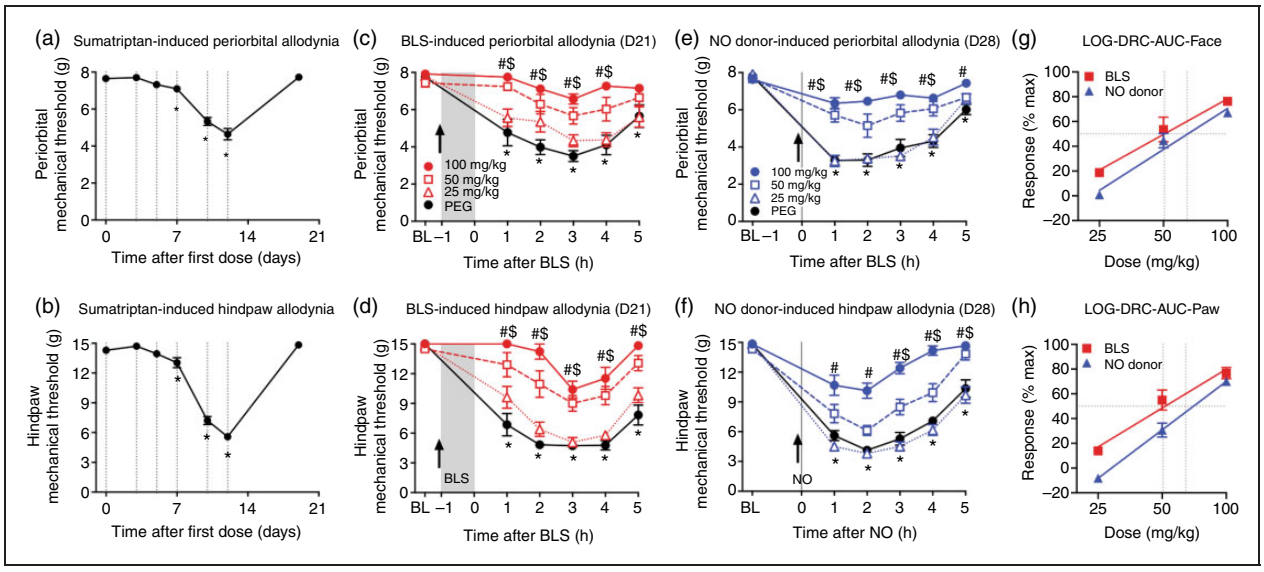

Figure 2. Female Sprague-Dawley rats received six oral doses of sumatriptan 10 mg/kg in water on days 0, 3, 5, 7, 10, and 12 (administration at dotted lines), and periorbital (a) and hindpaw (b) allodynia was measured for 19 days. Then, rats were exposed to bright light stress (BLS) on two consecutive days (days 20 and 21). On day 21, graded doses of ubrogepant were administered prior to BLS exposure, and periorbital (c) and hindpaw (d) allodynia was assessed hourly over 5 hours. On day 28, rats received graded doses of ubrogepant and were immediately exposed to a nitric oxide (NO) donor (sodium nitroprusside 3 mg/kg, i.p.); periorbital (e) and hindpaw (f) allodynia was assessed over 5 hours. Data are mean ± standard error of the mean; n = 6 rats/group. Ubrogepant dose-response curves were constructed from data in graphs (c) and (e) for the periorbital effects (g) and from graphs (d) and (f) for the hindpaw effects (h). LOG-DRC-AUC, log–dose-response curve–area under the curve.
*Significantly reduced withdrawal thresholds from the baseline.
#Significant difference between the PEG-treated group and the ubrogepant 100 mg/kg group.
$Significant difference between the PEG-treated group and the ubrogepant 50 mg/kg group.

hindpaw CA, as previously observed. In contrast, BLS-induced changes in sensory thresholds in the ubrogepant- and PEG- primed animals were minimal, and significantly less than those in the sumatriptan-primed animals (Figures 3(c) and (d), Table 1); there was no difference between the PEG- and ubrogepant-primed groups. Similarly, exposure of sumatriptan-primed rats to an NO donor on day 30 elicited significant CA, but NO donor-induced allodynia was significantly smaller in the PEG- and ubrogepant-primed rats, and there was no difference between the latter groups (Figures 3(e) and (f), Table 1).

Discussion

Frequent use or overuse of medications for acute treatment of headache can cause MOH – a significant burden for patients, a challenge for clinicians, and an impediment to successful treatment of some patients with migraine. Frequent or excessive use of acute medications may also be associated with gastrointestinal, hepatic, renal, and cardiovascular toxicity. Acute treatments for migraine that do not lead to MOH would fulfill a crucial unmet treatment need, reduce the development of MOH, and significantly contribute to improved patient care. At present, almost all of the therapies for acute treatment of migraine have been linked to increased frequency of headache and risk of development of MOH. Drugs of the "gepant" class, including ubrogepant, have demonstrated efficacy in the acute treatment of migraine (31–33). To date, however, it remains unknown whether these drugs promote MOH in vulnerable patients.

The MOH model used in these studies was based on a variation of the two-hit model of hyperalgesic priming in which vulnerability to a second stimulus is induced by treatment with a drug such as a triptan, an opiate, or a cannabinoid (17–19). In this model, the drugs initially produce a cephalic and extracephalic CA that suggests a state of central sensitization and is consistent with cephalic and extracephalic allodynia observed in patients during migraine attacks. After the drug-induced allodynia resolves, the "second hit" of provocative stimuli, including environmental stress induced by exposure to bright lights or challenge with an NO donor, promotes allodynia only in previously primed animals. This is indicative of latent sensitization of trigeminal and central pain modulatory pathways likely relevant to MOH.

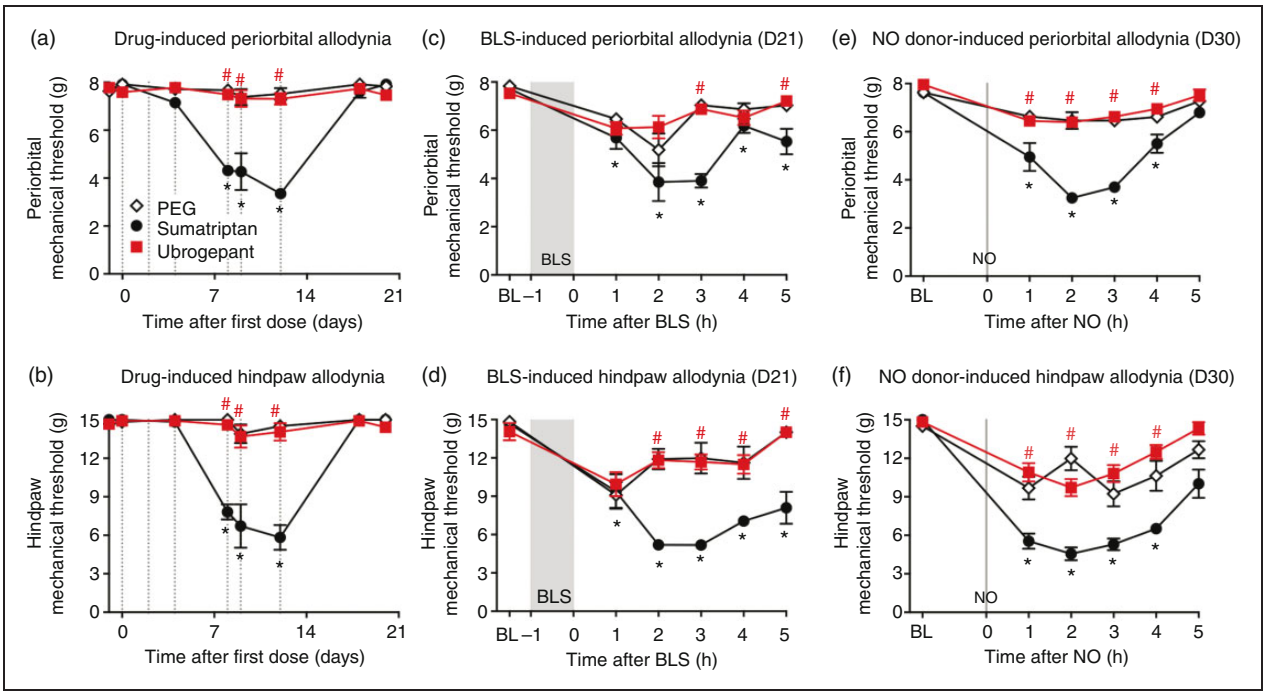

Figure 3. Female Sprague-Dawley rats received six oral doses of sumatriptan 10 mg/kg in water, polyethylene glycol (PEG; vehicle for ubrogepant), or ubrogepant 100 mg/kg (oral in PEG) on days 0, 2, 4, 8, 9, and 12 (administration at dotted lines), and periorbital (a) and hindpaw (b) allodynia was measured for 20 days. Rats were then exposed to bright light stress (BLS) on two consecutive days (days 20 and 21); periorbital (c) and hindpaw (d) allodynia was assessed hourly over 5 hours on the second day of BLS. On day 30, rats were exposed to a nitric oxide (NO) donor (sodium nitroprusside 3 mg/kg, i.p.), and periorbital (e) and hindpaw (f) allodynia was assessed over 5 hours. Repeated sumatriptan, but not ubrogepant, produced acute allodynia and BLS- and NO donor-induced allodynia, suggesting ubrogepant does not elicit latent sensitization on repeated dosing. Data are mean ± standard error of the mean; n = 6 rats in PEG group, n = 6 rats in sumatriptan group, n = 12 rats in ubrogepant group.
*Significantly reduced withdrawal thresholds from the baseline in the PEG-treated rats.
#Significant difference between the sumatriptan-treated group and both ubrogepant and PEG groups.

In this study, both ubrogepant and sumatriptan effectively reversed stress-induced allodynia in a rat model of MOH induced by sumatriptan priming. This finding is consistent with the clinical efficacy of these drugs in acute treatment of migraine, including migraine attacks in patients with MOH (34). However, the present studies show that, unlike sumatriptan, repeated administration of ubrogepant at a dose that effectively reverses stress- and NO donor-induced allodynia in MOH rats does not promote CA or elicit latent sensitization. If established clinically, the absence of MOH may become a defining hallmark of this class of medications, allowing their potential use at a frequency higher than that of currently available medications.

The absence of central sensitization following priming with repeated administration of ubrogepant is likely attributable to the antagonist effects of ubrogepant on CGRP receptors. Most MOH-producing drugs, including triptans, opiates, and cannabinoids, are receptor agonists; others, such as acetaminophen and barbiturates, have unclear mechanisms. Agonist drugs directly activate their respective receptors and mimic, to some degree, the effects of endogenous neurotransmitters. In this regard, it is relevant to note that agonist drugs bypass the physiologic regulation of endogenous neurotransmitters that occur in specific circuits in response to physiologic demands (35). Additionally, with extended use, agonist drugs can promote neural adaptations that may underlie central sensitization and increased vulnerability to migraine triggers (18). It has been demonstrated that, following chronic treatment in cell cultures, opioid agonists promote neuronal hyperexcitability, which suggests that some neural adaptations observed *in vivo* can be caused by direct cellular effects (36). Additionally, it may be hypothesized that MOH from agonist drugs may result, in part, from well-known desensitization and/or down-regulation of receptors involved in drug responses (18,37) resulting in net increased excitability of circuits relevant to a form of "withdrawal" (38,39). In contrast, antagonists are known to produce upregulation of receptors (37). Furthermore, unlike agonists, antagonists by definition are only effective in activated

Table 1. Summary of statistical analyses, *p*-values, and *F* ratios for Figures 1–3.

Figure	Analysis	Time	Treatment	Interaction
1(a)	1-way ANOVA	$p < 0.0001$	–	–
		$F_{6,77} = 25.49$	–	–
1(b)	1-way ANOVA	$p < 0.001$	–	–
		$F_{6,77} = 128.7$	–	–
1(c)	2-way RM ANOVA	$p < 0.0001$	$p = 0.0115$	$p = 0.0241$
		$F_{5,50} = 16.2$	$F_{1,10} = 9.53$	$F_{5,50} = 2.86$
1(d)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p = 0.0078$
		$F_{5,50} = 32.9$	$F_{1,10} = 41.8$	$F_{5,50} = 3.57$
1(e)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
		$F_{4,40} = 38.9$	$F_{1,10} = 79.5$	$F_{4,40} = 11.5$
1(f)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
		$F_{4,40} = 79.2$	$F_{1,10} = 141$	$F_{4,40} = 19.4$
2(a)	1-way ANOVA	$p < 0.0001$	–	–
		$F_{6,161} = 55.8$	–	–
2 (b)	1-way ANOVA	$p < 0.0001$	–	–
		$F_{6,161} = 161$	–	–
2 (c)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p = 0.0061$
		$F_{5,100} = 22.5$	$F_{3,20} = 39.5$	$F_{15,100} = 2.36$
2 (d)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
		$F_{5,100} = 62.9$	$F_{3,20} = 50.9$	$F_{15,100} = 4.87$
2 (e)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
		$F_{5,100} = 69.7$	$F_{3,20} = 74.8$	$F_{15,100} = 5.66$
2 (f)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
		$F_{5,100} = 140$	$F_{3,20} = 74.8$	$F_{15,100} = 6.34$
3 (a)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
		$F_{7,147} = 26.8$	$F_{2,21} = 82.0$	$F_{14,147} = 16.3$
3 (b)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
		$F_{7,147} = 29.9$	$F_{2,21} = 66.8$	$F_{14,147} = 18.5$
3 (c)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p = 0.0003$
		$F_{5,105} = 16.9$	$F_{2,21} = 26.7$	$F_{10,105} = 3.67$
3 (d)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
		$F_{5,105} = 17.6$	$F_{2,21} = 41.2$	$F_{10,105} = 4.63$
3 (e)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
		$F_{4,84} = 39.8$	$F_{2,21} = 124$	$F_{8,84} = 7.13$
3 (f)	2-way RM ANOVA	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
		$F_{4,84} = 41.1$	$F_{2,21} = 74.1$	$F_{8,84} = 5.35$

ANOVA: analysis of variance; RM: repeated measures.

physiological synapses to block endogenous neurotransmission. These observations of lack of central sensitization in the preclinical model are consistent with clinical data showing that CGRP antagonists are effective in reducing the frequency of migraine (40) and that erenumab, a monoclonal antibody targeting the CGRP receptor, does not lead to worsening of headaches (41), a feature that is a key characteristic of MOH. However, it remains to be determined if very long-term use of ubrogepant would increase the risk of MOH.

Preclinical rodent models of migraine, and MOH in particular, have found that CGRP is increased in jugular blood (18,42) and that stress- or NO donor-induced allodynia in primed rats is effectively blocked by NSAIDs, triptans, cannabinoids, and pretreatment with a CGRP monoclonal antibody (17,18,21,43). Ubrogepant is a clinically effective small-molecule CGRP receptor antagonist (31,33), but its precise site of action remains unclear. CGRP receptors are expressed peripherally on cranial blood vessels, glial cells, and neurons, including Aδ fibers in the trigeminal ganglion (44). Centrally, these receptors can be found on terminals of trigeminal afferents, in the brain stem, cerebellum, and cerebral hemispheres (45). Although CGRP receptors at all these sites may be relevant to migraine, ubrogepant poorly penetrates the blood-brain barrier, suggesting that the major site of action is likely on the periphery (46). This conclusion is consistent with studies that have demonstrated that peripheral CGRP can trigger migraine pain (47) and that

monoclonal antibodies that do not significantly penetrate the blood-brain barrier can effectively reduce the frequency of migraine attacks (48).

Preclinical evidence suggests that CGRP may be crucial in the pathogenesis of MOH (18). Therefore, ubrogepant may be used to block CGRP signaling and thereby prevent the development of central sensitization associated with MOH and possibly reverse established central sensitization in patients with chronic migraine and/or MOH. Indeed, some emerging evidence indicates that CGRP-receptor antibodies are effective in reducing migraine frequency and acute medication use in patients with chronic migraine who also overuse acute medications, including those patients who have not responded to previous preventive treatments (49,50). Atogepant, another small-molecule oral CGRP receptor antagonist, is being developed for the preventive treatment of migraine based on a phase 2b/3a randomized placebo-controlled study demonstrating efficacy in reducing headache frequency when used daily (51). Rimegepant given at least every other day plus as needed for a period of 3 months led to decreases from baseline in migraine days per month (52). Therefore, drugs in the gepant family may also act preventively.

These findings may be interpreted as supporting a conclusion of no risk of MOH but, as with all animal models, extrapolating these findings to humans requires caution. Mechanisms promoting migraine pain in rodents and humans may be different, and the timelines and doses of the drugs used are significantly different and are linked in part to differences in metabolism between species. Additionally, the timelines and doses required to elicit vulnerability from presumed sensitized states in rodents are undoubtedly different from those that may be required in humans. We note that a major limitation of this study was that it was conducted for unavoidable reasons without blinding of the experimenter. However, outcomes of the data with sumatriptan were consistent with our previously published studies, which were conducted under blinded conditions (18,21,35). Additionally, we note that this study was conducted with female rats because MOH is more common among women (1); experiments are needed to establish effects in male rodents. Notwithstanding these noted limitations, however, the data suggest ubrogepant may be uniquely different from other acute treatments in its lack of potential for inducing MOH.

Conclusions

The outcomes of these experiments are twofold. First, the study demonstrates that, similar to sumatriptan, ubrogepant significantly blocks stress-induced allodynia in a rat model of cephalic pain relevant to MOH. This finding is consistent with a CGRP-dependent mechanism previously established in this model and demonstrates that CGRP receptors can be appropriately engaged by ubrogepant in rodents. Second, the study shows that, unlike sumatriptan, a dose of ubrogepant that is repeatedly administered and that engages the CGRP receptor does not elicit cephalic and hindpaw allodynia and does not elicit latent sensitization in rats. Collectively, the data suggest that repeated treatment with ubrogepant is unlikely to produce the neural adaptations that underlie MOH. These findings contrast with findings that triptans, opiates, cannabinoids, and other drugs used for the acute treatment of migraine lead to latent sensitization, as revealed by provocative challenge with environmental stress. Ubrogepant may therefore be a highly promising new medication for the acute treatment of migraine, especially in patients who have a history of MOH or at risk of developing MOH, including those with frequent migraine attacks or chronic migraine.

Key findings

- Ubrogepant administration resulted in a dose-related reversal of allodynia in rats previously primed with sumatriptan.
- Repeated administration of ubrogepant resulted in neither allodynia nor latent sensitization in rats.
- Drugs of the gepant class are unlikely to lead to medication overuse headache and are potentially viable medications for acute treatment of migraine regardless of headache frequency.

Declaration of conflicting interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: EN, SB, and JO report no conflicts of interest.

DWD reports the following conflicts. Personal fees: AEON, Alder BioPharmaceuticals, Allergan, Amgen, Amzak Health, Association of Translational Medicine, Autonomic Technologies, Axsome, Biohaven, Charleston Laboratories, Clexio, Daniel Edelman Inc., Dr Reddy's Laboratories/Promius, electroCore LLC, Eli Lilly, eNeura,

Equinox, Foresite Capital, Impel, Ipsen, Neurolief, Nocira, Novartis, Oppenheimer, Pieris, PSL Group Services, Revance, Salvia, Satsuma, Sun Pharma (India), Supernus, Teva, Theranica, University Health Network, Upjohn (Division of Pfizer), Vedanta, WL Gore, XoC, Zosano, and ZP Opco; Speaking fees: Amgen, Eli Lilly, Lundbeck, and Novartis Canada; CME fees or royalty payments: Academy for Continued Healthcare Learning, Cambridge University Press, Catamount, Chameleon, Global Access Meetings, Global Life Sciences, Global Scientific Communications, Haymarket, HealthLogix, Medicom Worldwide, MedLogix Communications, Mednet, Miller Medical, Oxford University Press, PeerView, Universal Meeting Management, UpToDate (Elsevier), WebMD Health/Medscape, and Wolters Kluwer Health; Stock options: Aural Analytics, Epien, Healint, King-Devick Technologies, Matterhorn, Nocira, Ontologics, Precon Health, Second Opinion/Mobile Health, and Theranica; Consulting without fee: Aural Analytics, Epien, Healint, Second Opinion/Mobile Health; Board of Directors: Epien, King-Devick Technologies, Matterhorn, Ontologics, and Precon Health; Patent: 17189376.1-1466:vTitle: Botulinum Toxin Dosage Regimen for Chronic Migraine Prophylaxis without fee; Research funding: American Migraine Foundation, Henry Jackson Foundation, Patient-Centered Outcomes Research Institute (PCORI), and US Department of Defense; professional society fees or reimbursement for travel: American Academy of Neurology, American Brain Foundation, American Headache Society, American Migraine Foundation, Canadian Headache Society, and International Headache Society.

PB is an employee of Allergan plc; FP has served as a consultant or received research funding from Acadia, Allergan, Amgen, Blackthorn, Eli Lilly, Hoba, Ipsen, Nektar, Proximagen, SiteOne Therapeutics, Teva, and Voyager, and has served as a founder of Catalina Pharma, and Scientific Advisory Board Regulonix.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by a grant from Allergan plc (FP). Editorial support was provided by Peloton Advantage, LLC, an OPEN Health company (Parsippany, NJ), and funded by Allergan plc.

ORCID iD

Edita Navratilova https://orcid.org/0000-0002-1497-125X

References

1. Bigal ME, Rapoport AM, Sheftell FD, et al. Transformed migraine and medication overuse in a tertiary headache centre–clinical characteristics and treatment outcomes. *Cephalalgia* 2004; 24: 483–490.
2. Bigal ME, Serrano D, Buse D, et al. Acute migraine medications and evolution from episodic to chronic migraine: A longitudinal population-based study. *Headache* 2008; 48: 1157–1168.
3. Suh GI, Park JW and Shin HE. Differences in clinical features and disability according to the frequency of medication use in patients with chronic migraine. *J Clin Neurol* 2012; 8: 198–203.
4. Olesen J. International classification of headache disorders. *Lancet Neurol* 2018; 17: 396–397.
5. Headache Classification Committee of the International Headache Society. The International Classification of Headache Disorders, 3rd edition. *Cephalalgia* 2018; 38: 1–211.
6. Aaseth K, Grande RB, Kvaerner KJ, et al. Prevalence of secondary chronic headaches in a population-based sample of 30–44-year-old persons. The Akershus study of chronic headache. *Cephalalgia* 2008; 28: 705–713.
7. Colas R, Munoz P, Temprano R, et al. Chronic daily headache with analgesic overuse: epidemiology and impact on quality of life. *Neurology* 2004; 62: 1338–1342.
8. Jonsson P, Hedenrud T and Linde M. Epidemiology of medication overuse headache in the general Swedish population. *Cephalalgia* 2011; 31: 1015–1022.
9. Lu SR, Fuh JL, Chen WT, et al. Chronic daily headache in Taipei, Taiwan: Prevalence, follow-up and outcome predictors. *Cephalalgia* 2001; 21: 980–986.
10. Zwart JA, Dyb G, Hagen K, et al. Analgesic overuse among subjects with headache, neck, and low-back pain. *Neurology* 2004; 62: 1540–1544.
11. James SL, Abate D, Abate KH, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018; 392: 1789–1858.
12. Thorlund K, Sun-Edelstein C, Druyts E, et al. Risk of medication overuse headache across classes of treatments for acute migraine. *J Headache Pain* 2016; 17: 107.
13. Grazzi L, Grignani E, D'Amico D, et al. Is medication overuse drug specific or not? Data from a review of published literature and from an original study on Italian MOH patients. *Curr Pain Headache Rep* 2018; 22: 71.
14. Wang SJ, Fuh JL, Lu SR, et al. Quality of life differs among headache diagnoses: Analysis of SF-36 survey in 901 headache patients. *Pain* 2001; 89: 285–292.
15. Meletiche DM, Lofland JH and Young WB. Quality-of-life differences between patients with episodic and transformed migraine. *Headache* 2001; 41: 573–578.
16. Raggi A, Schiavolin S, Leonardi M, et al. Chronic migraine with medication overuse: Association between disability and quality of life measures, and impact of disease on patients' lives. *J Neurol Sci* 2015; 348: 60–66.
17. Kopruszinski CM, Navratilova E, Vagnerova B, et al. Cannabinoids induce latent sensitization in a preclinical model of medication overuse headache. *Cephalalgia* 2020; 40: 68–78.
18. De Felice M, Ossipov MH, Wang R, et al. Triptan-induced latent sensitization: A possible basis for medication overuse headache. *Ann Neurol* 2010; 67: 325–337.

19. Nation KM, Dodick DW, Navratilova E, et al. Sustained exposure to acute migraine medications combined with repeated noxious stimulation dysregulates descending pain modulatory circuits: Relevance to medication overuse headache. *Cephalalgia* 2019; 39: 617–625.
20. Chaplan SR, Bach FW, Pogrel JW, et al. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods* 1994; 53: 55–63.
21. Kopruszinski CM, Xie JY, Eyde NM, et al. Prevention of stress- or nitric oxide donor-induced medication overuse headache by a calcitonin gene-related peptide antibody in rodents. *Cephalalgia* 2017; 37: 560–570.
22. Burstein R, Yarnitsky D, Goor-Aryeh I, et al. An association between migraine and cutaneous allodynia. *Ann Neurol* 2000; 47: 614–624.
23. Mathew NT, Kailasam J and Seifert T. Clinical recognition of allodynia in migraine. *Neurology* 2004; 63: 848–852.
24. Ashkenazi A, Sholtzow M, Shaw JW, et al. Identifying cutaneous allodynia in chronic migraine using a practical clinical method. *Cephalalgia* 2007; 27: 111–117.
25. Schwedt TJ, Alam A, Reed ML, et al. Factors associated with acute medication overuse in people with migraine: Results from the 2017 migraine in America symptoms and treatment (MAST) study. *J Headache Pain* 2018; 19: 38.
26. Boyer N, Dallel R, Artola A, et al. General trigeminospinal central sensitization and impaired descending pain inhibitory controls contribute to migraine progression. *Pain* 2014; 155: 1196–1205.
27. Oshinsky ML and Gomonchareonsiri S. Episodic dural stimulation in awake rats: A model for recurrent headache. *Headache* 2007; 47: 1026–1036.
28. Moye LS, Tipton AF, Dripps I, et al. Delta opioid receptor agonists are effective for multiple types of headache disorders. *Neuropharmacology* 2019; 148: 77–86.
29. Tepper SJ. History and review of anti-calcitonin gene-related peptide (CGRP) therapies: From translational research to treatment. *Headache* 2018; 58: 238–275.
30. Edvinsson L. The trigeminovascular pathway: Role of CGRP and CGRP receptors in migraine. *Headache* 2017; 57: 47–55.
31. Dodick DW, Lipton RB, Ailani J, et al. Ubrogepant for the treatment of migraine. *N Engl J Med* 2019; 381: 2230–2241.
32. Goadsby PJ. Primary headache disorders: Five new things. *Neurology Clin Pract* 2019; 9: 233–240.
33. Lipton RB, Dodick DW, Ailani J, et al. Effect of ubrogepant versus placebo on pain and the most bothersome associated symptom in the acute treatment of migraine: The ACHIEVE II randomized clinical trial. *JAMA* 2019; 322: 1887–1898.
34. Tepper SJ. Medication-overuse headache. *Continuum (Minneapolis, Minn)* 2012; 18: 807–822.
35. Xie JY, De Felice M, Kopruszinski CM, et al. Kappa opioid receptor antagonists: A possible new class of therapeutics for migraine prevention. *Cephalalgia* 2017; 37: 780–794.
36. Yue X, Tumati S, Navratilova E, et al. Sustained morphine treatment augments basal CGRP release from cultured primary sensory neurons in a Raf-1 dependent manner. *Eur J Pharmacol* 2008; 584: 272–277.
37. van Hoogstraten WS and MaassenVanDenBrink A. The need for new acutely acting antimigraine drugs: Moving safely outside acute medication overuse. *J Headache Pain* 2019; 20: 54.
38. Cheung V, Amoozegar F and Dilli E. Medication overuse headache. *Curr Neurol Neurosci Rep* 2015; 15: 509.
39. Hitomi S, Kross K, Kurose M, et al. Activation of dura-sensitive trigeminal neurons and increased c-Fos protein induced by morphine withdrawal in the rostral ventromedial medulla. *Cephalalgia* 2017; 37: 407–417.
40. Israel H, Neeb L and Reuter U. CGRP monoclonal antibodies for the preventative treatment of migraine. *Curr Pain Headache Rep* 2018; 22: 38.
41. Schwedt T, Reuter U, Tepper S, et al. Early onset of efficacy with erenumab in patients with episodic and chronic migraine. *J Headache Pain* 2018; 19: 92.
42. Wang X, Fang Y, Liang J, et al. Selective inhibition of 5-HT7 receptor reduces CGRP release in an experimental model for migraine. *Headache* 2010; 50: 579–587.
43. Navratilova E, Rau J, Oyarzo J, et al. CGRP-dependent and independent mechanisms of acute and persistent post-traumatic headache following mild traumatic brain injury in mice. *Cephalalgia* 2019; 39: 1762–1775.
44. Edvinsson JCA, Warfvinge K, Krause DN, et al. C-fibers may modulate adjacent Adelta-fibers through axon-axon CGRP signaling at nodes of Ranvier in the trigeminal system. *J Headache Pain* 2019; 20: 105.
45. Digre KB. What's new in the treatment of migraine? *J Neuroophthalmol* 2019; 39: 352–359.
46. Moore E, Fraley ME, Bell IM, et al. Characterization of ubrogepant: A potent and selective antagonist of the human calcitonin gene-related peptide receptor. *J Pharmacol Exp Ther* 2020; 373: 160–166.
47. Lassen LH, Haderslev PA, Jacobsen VB, et al. CGRP may play a causative role in migraine. *Cephalalgia* 2002; 22: 54–61.
48. Olesen J, Diener HC, Husstedt IW, et al. Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine. *N Engl J Med* 2004; 350: 1104–1110.
49. Tepper SJ, Diener HC, Ashina M, et al. Erenumab in chronic migraine with medication overuse: Subgroup analysis of a randomized trial. *Neurology* 2019; 92: e2309–e2320.
50. Dodick D, Tepper SJ, Diener HC, et al. Efficacy of erenumab in chronic migraine patients with medication overuse and prior preventive treatment failure [abstract]. *Neurology* 2019; 92: S38.002.
51. Goadsby PJ, Dodick DW, Trugman JM, et al. Orally administered atogepant was efficacious, safe, and tolerable for the prevention of migraine: Results from a phase 2b/3 study [abstract]. *Neurology* 2019: 92: S17.001.
52. Lipton RB, Berman G, Kudrow D, et al. Long-term, open-label safety study of rimegepant 75 mg for the treatment of migraine (study 201): Interim analysis of safety and exploratory efficacy (abstract P235LB). *Headache* 2019; 59: 175.