Purdue Pharma L.P.

6 Cedar Brook Drive
Cranbury, NJ 08512-3636
www.purduepharma.com

Name of journal: World Journal of Gastroenterology

Manuscript NO.: 33327

Column: Basic Study

May 29, 2017

Dear Yuan Qi:

We are pleased to submit our revised manuscript entitled, "*The Pharmacological Evaluation of NSAID-Induced Gastropathy as a "Translatable" Model of Referred Visceral Hypersensitivity"* to BPG.

We can confirm the work has not been submitted for publication in whole or in part elsewhere and all authors listed on the title page have approved the manuscript that is being submitted.

On behalf of all authors, I thank you and the two reviewers for their time and insightful comments and suggestions which we have addressed in the manuscript as described below. We agree that all have significantly improved the manuscript.

In this regard, all revised sections are highlighted in red in the manuscript itself. Additionally, we have included our comments for revision in the text below.

Sincerely,

Michele Hummel PhD

Reviewer #1- However, the manuscript is not suitable for publication at this stage unless the below questions are clarified.

1. In the figure legends, what do "*P< 0.05" and "#P< 0.05" refer to? *For all data figures, #P< 0.05 vs vehicle and *P< 0.05 vs indomethacin.*

2. In reference to the interpretation of the effects of morphine in the results of opioid receptors, I do not think the description of the figure 2A is appropriate, and the time is needed to be added. In addition, the inappropriate description of asimadoline is similar to that of morphine in the figure 2B.

*Figure 2A (morphine): Results showed successful ulcer model development both 4 and 24-hours post-indomethacin dosing (#P< 0.05 vs vehicle), and that morphine attenuated the indomethacin-induced visceral hypersensitivity with a minimal effective dose (MED) equal to 10 mg/kg. Although there was a main effect of treatment (F (4,54)= 36, *P< 0.05 vs indomethacin) and a significant interaction with time (F(8,79)= 21, *P< 0.05 vs indomethacin), the effect morphine elicited on the pain behavior was only noted 2 hours post-dosing (4 hours post-indomethacin) for each of the higher doses tested, 10-30 mg/kg. Morphine was not efficacious in the assessment conducted 24- hours post-indomethacin dosing. Nonetheless, the short-lived efficacy is consistent with the half-life of this drug.*

Figure 2B (asimadoline): Subsequently, we next examined the effect asimadoline, a selective kappa opioid receptor (KOR) agonist, had in this pain model as well. Results showed consistent model development both 4 and 24- hours post-indomethacin dosing (#P< 0.05 vs vehicle).

3. The list of references is not in our style, and the authors should increase some recent references.

The reference style has been corrected as per journal instructions. Please see WORD version of manuscript.

A number of references have been added. These include the following:

15. Matsui H, Shimokawa O, Kaneko T, Nagano Y, Rai K, Hyodo I. The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries in stomach and small intestine. J Clin Biochem Nutr 2011; 48(2): 107-111 [PMID: 21373261 DOI: 10.3164/jcbn.10-79]

20. Farmer A, Aziz Q. Gut pain and visceral hypersensitivity. Br J Pain 2013; 7(1): 39-47 [PMID: 26516496 DOI: 10.1177/2049463713479229]

34. Fox-Orenstein AE. New and emerging therapies for the treatment of irritable bowel syndrome: an update for gastroenterologists. Therap Adv Gastroenterol 2016; 9(3): 354-375 [PMID: 27134665 DOI: 10.1177/1756283X16633050]

42. Camilleri M. Novel pharmacology: asimadoline, a kappa opioid agonist, and visceral sensation. Neurogastroenterol Motil 2008; 20(9): 971-979 [PMID: 18715494 DOI: 10.1111/j.1365-2982.2008.01183.x]

51. Akbar A, Yiangou Y, Facer P, Walters JR, Anand P, Ghosh S. Increased capsaicin receptor TRPV1-expressing sensory fibres in irritable bowel syndrome and their correlation with abdominal pain. Gut 2008; 57: 923-929 [PMID: 18252749 DOI: 10.1136/gut.2007.138982]

60. Liu S, Cheng XY, Fen W, Liu CF. Acid-sensing ion channels: potential therapeutic targets for neurological diseases. Transl Neurodegener 2015; 4(10): 1-8 [PMID: 26029363 DOI: 10.1186/s40035-015-0031-3]

68. Mayer EA, Bradesi S, Chang L, Spiegel BMR, Bueller JA Naliboff BD. Functional GI disorders: from animal models to drug development. Gut 2008; 57(3): 384–404 [PMID: 17965064 DOI: 10.1136/gut.2006.101675]

4. Some abbreviations are not annotated for the first time, such as "Similar to other GI disorders" in page 2. In addition, the abbreviations and the original word are confusing in the text and there is a need for more uniform language, for example guanylate cyclase C in page 4, 10, 18, 20, 24, and GC-C in page 15, 26.

These corrections have been made in the WORD document. Please refer to each of the cited pages above. All are indicated in red text.

Reviewer #2- What are the study limitations? *We have stated the limitations associated with this study in the concluding paragraph in the Discussion section.*

...Furthermore, while we think this model is well-suited for drug discovery namely because of its ease and its reliability in performing stringent pharmacological evaluation (i.e. dose-response data), it does have its limitations. For one, it is an acute model of visceral pain. While acute visceral pain is burdensome, the unmet need lies with the undulating hypersensitivity that persists from chronicity like in IBS. Also, most discovery groups perform their pain studies in rats, so consistency with regard to species selection does differ as this model was validated in mice. Lastly, functional GI disorders represent a heterogeneous group of disorders that include genetic and environmental contributors. Notably, these factors are very difficult to recapitulate in animals. While these limitations are well-recognized, together with its clinical relevance, we believe that the NSAID-induced gastropathy model may help uncover additional targets contributing to persistent GI pain of ill-defined etiology and further advance future drug discovery efforts for this unmet need.

Scientific Editor:

Word version provided.

Author Contributions: *Hummel M and Whiteside G conceptualized study design, analysis, and interpretation of study results. Hummel M wrote the manuscript. Knappenberger T collected and analyzed the data. Reilly M prepared the figures depicting the data.*

Institutional Review Board Statement*: not applicable*

Conflict-of-Interest Statement: *There are no conflicts of interest with the authors.*

Data Sharing Statement: *No additional data are available*.

Biostatistics Statement: *The statistical methods of this study were reviewed by Salvatore Colucci, a biomedical statistician employed by Purdue Pharma L.P.*

Abstract:
AIM *To evaluate whether NSAID-induced gastropathy is a clinically predictive model of referred visceral hypersensitivity.*
METHODS *Gastric ulcer pain was induced by the oral administration of indomethacin to male, CD1 mice (n=10/group) and then assessed by measuring referred abdominal hypersensitivity to tactile application. A diverse range of pharmacological mechanisms contributing to the pain were subsequently investigated. These mechanisms included: transient receptor potential (TRP), sodium and acid-sensing ion channels (ASICs) as well as opioid receptors and guanylate cyclase C (GC-C).*
RESULTS *Results showed that two opioids and a GC-C agonist, morphine, asimadoline & linaclotide, respectively, the TRP antagonists, AMG9810 & HC-030031 and the sodium channel blocker, carbamazepine, elicited a dose- and/or time-dependent attenuation of referred visceral hypersensitivity, while the ASIC blocker, amiloride, was ineffective at all doses tested.*
CONCLUSION *Together, these findings implicate opioid receptors, GC-C, and sodium and TRP channel activation as possible mechanisms associated with visceral hypersensitivity. More importantly, these findings also validate NSAID-induced gastropathy as a sensitive and clinically predictive mouse model suitable for assessing novel molecules with potential pain-attenuating properties.*

Key Words: *visceral hypersensitivity; pain; translation; guanylate cyclase C; TRP channel; NSAID*

Core Tip: *Recently, standard animal models of pain have been vehemently challenged for their inability to successfully predict human clinical outcomes. Further, few animal models have been represented with reasonable translational value for conditions presenting with visceral pain. NSAID-induced gastropathy represents a translatable model of visceral*

hypersensitivity in which several pain targets have demonstrated reliable sensitivity when assayed. Further, this model is robust enough that proper pharmacological evaluation can be conducted. Overall, this model has the potential to efficiently triage molecules with pain-attenuating properties for their utility in GI disorders that include pain as a hallmark symptom.

COMMENTS

Background*: Clinically, NSAIDS like ibuprofen and indomethacin have been show to produce profound ulcerative effects on the GI tract. These untoward effects can be championed for their translational value in developing and validating relevant preclinical animal models that may enable future drug discovery efforts.*

Research Frontiers*: Visceral pain represents an unmet medical need. While some novel drugs like linaclotide have demonstrated favorable clinical success, there are many patients with ill-defined functional GI disorders that are underserved.*

Innovations and breakthroughs: *In this study, the authors validated a visceral model of pain for preclinical evaluation that has translational significance and value. The model provides a higher-throughput behavioral approach for assessing the acute pain alleviating effects of a diverse range of pharmacological targets.*

Applications: *The authors confirmed that the NSAID-induced gastopathy model together with a mechanical endpoint can be used to triage the analgesic effects of novel compounds for visceral pain.*

Terminology: *Visceral hypersensitivity is pain that can be very non-specific and diffuse that results from the activation of nociceptors in the thoracic, pelvic, or abdominal area.*