Author's response to reviews

Title: Resolvin D1 reverses chronic pancreatitis-induced mechanical allodynia, phosphorylation of NMDA receptors, and cytokines expression in the thoracic spinal dorsal horn

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Author's response to reviews: see over
Cover Letter

Dear Dr Timothy Shipley, Executive Editor of BMC Gastroenterology,

Thank you very much for the comments on our manuscript “Resolvin D1 reverses chronic pancreatitis-induced mechanical allodynia, phosphorylation of NMDA receptors, and cytokines expression in the thoracic spinal dorsal horn” (MS: 1448786458725205). The comments have helped us improve our manuscript. We have revised this manuscript according to the reviewers’ comments. The changes in the manuscript were indicated with red color.

We hope all these changes could work and we hope our manuscript can be accepted by your journal.

Best wishes,

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Point-by-point response to the concerns

Reviewer1:

In their current study, Feng et al. investigated the potential analgesic effect of resolvin, an omega-3-polyunsaturated fatty acid-derived molecule, in experimental chronic pancreatitis (CP) in rats. Intrathecal administration of resolvin (termed RvD) reduced mechanical allodynia of rats with TNBS-induced CP, reduced the amount of IL6, TNFalpha and IL-1beta mRNA in the thoracic spinal cord, and reversed the phosphorylation of NMDA receptor subtypes NR1 and NR2B. Based on these results,
the authors concluded that resolvin may be a novel anti-inflammatory therapeutic for the treatment of CP-associated pain.

Overall, the study conveys a straightforward message and investigated a molecule class of high interest to treat inflammatory pain. However, despite these clear messages within the manuscript, the provided data remain very superficial, and the applied methodology is insufficient to support the proposed analgesic effect of resolvin. In particular, I have the following major and minor comments:

1) Assessment of pain in murine experimental models should contain true parameters of pain-related “behavior” and not be limited to evaluation of mechanical allodynia, which represents a single facet of the complex “pain phenomenon”. In line with the majority of experimental pain studies, the authors should make use of additional tests to assess CP-related pain behavior (e.g. open field test, elevated maze or resistance-to-capture, etc.). Without these, the authors should refrain from referring to the effects of resolvin upon “pain behavior” in the manuscript.

Response: Thanks for your good suggestion. We have performed open field test and elevated plus maze. We found that neither CP nor RvD1 treatment could affect anxiety-like behavior. CP or RvD1 treatment could not affect free locomotor and exploration of rats.

2) Although the manuscript stems from a group with experience in histological assessment of intrinsic cells of the spinal cord in murine CP-induced pain, the study in no way touches the affected cell type by resolvin treatment. Which cells in the spinal cord lose their NMDA receptor phosphorylation upon resolvin treatment? This can be easily demonstrated by phosphor-NR1/2B-specific antibodies via immunohisto/cytochemistry.

Response: Thanks for your good suggestion. Many studies have confirmed that phosphor-NR1/2B could be detected in spinal neurons in different pain models. In addition, non-neuronal expression of phosphor-NR1/2B could also be found, but in an extremely low level. We tried many times for the double staining to detect the phosphor-NR1/2B. However, no good data could be obtained after many trials. That is why we asked for a delay for the revision. If you insist that this experiment should be done, we may purchase some new antibodies and try our best to finish it.

3) The demonstrated alterations in the level of pro-inflammatory cytokines in the spinal cord vary between 1-2-fold, which, although statistically significant, are very unlikely to represent biologically relevant alterations. Furthermore, the assessment of these cytokines at RNA level does not contribute significantly towards understanding the actual modulation of inflammation in the spinal cord through resolvin, which
seems to be the leading mechanism in resolvin-mediated analgesia. Therefore, the investigators should measure the levels of these cytokines at protein level.

Response: Thank you for your suggestions. We have performed Enzyme-linked immunosorbent assay (ELISA) according to your comments. In accordance to the real-time PCR data, the protein levels of inflammatory cytokines were also significantly modulated by CP and RvD1 administration.

4) In the whole manuscript, the administered drug is termed “resolvin/Rvd”, which, however, normally designates a “class” of resolvins and contains several resolvin subtypes. Did the authors administer one or more resolvin subtypes in their experiments? This remains totally unclear.

Response: In the present study, we only administered resolvin D1 (7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid). To make it clear, we revised RvD to RvD1.

Minor comments:
1) How were the dosages for Rvd chosen in the study? This is particularly interesting seeing the large leap between the two applied dosages (10 vs. 500 ng).

Response: In the present study, the dosages were chosen according to previous reports. In addition, to evaluate the potential dose-related effect, we used 3 dosages (10, 50 and 500 ng). We tested behavioral effect with these 3 doses but in the biochemical analysis, we only evaluated those rats treated with the highest (500 ng) and lowest (10 ng) doses of RvD1.

2) In the methods, the authors specify that pancreatic inflammation was histologically assessed. Were there also any differences in the severity of intrapancreatic inflammation between treated vs. non-treated groups? Furthermore, can resolvin also be detected in the serum and reach distant targets other than spinal cord (e.g. pancreas)?

Response: Thank you for the comments. We have revised the end of the first part of discussion section according to your comments. We did not find any differences in the severity of intrapancreatic inflammation between treated vs. non-treated groups. We administered RvD1 intrathecally in very low doses, which could not easily affect inflammation in the pancreas, especially when we injected RvD1 at 5w after induction of inflammation. That is why we believe that the analgesic effect of RvD1 is directly through spinal dorsal horn. In addition, so far we have no technique to detect resolvin expression. We agree with you that this is an interesting issue and we will develop some methods in the future.
studies.

3) The location of NR1 and NR2B expression should be mentioned in the abstract.
Response: We are sorry for this comment. Please see above.

Reviewer2:
In this study, Feng et al observed that intrathecal injection of Resolvin D1 could dose-dependently attenuate pain of CP. They also showed that intrathecal injection of RvD could remarkably reverse NMDA receptor phosphorylation and cytokine expressions in the spinal dorsal horn of CP rats. These findings are interesting and important and may provide new insights into mechanism and treatment of pain in patients of chronic pancreatitis. The methods are appropriately described, the experiments are well designed, and the data is sound and convincing. The discussion and conclusions are well balanced and adequately supported by the data. However, there are a few errors in grammar and spelling that need to be corrected. Figure 3 and 4 Legends: Please provide more explanatory information in the legend.
Response: Thanks for your good suggestion. We have revised the manuscript according to your comments.