Author’s response to reviews

Title: Remifentanil ameliorates intestinal ischemia-reperfusion injury

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Author’s response to reviews: see over
To Mark Andrew Cardinez,

BMC Gastroenterology

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Dear Dr Cardinez,

Please find enclosed the revised version of our manuscript "Remifentanil ameliorates intestinal ischemia-reperfusion injury" (MS: 1667756164797550) by Steven SC Cho, Ina Rudloff, Philip J Berger, Michael G Irwin, Marcel F Nold, Wei Cheng and myself. We have substantially revised our manuscript and have addressed all of the Reviewers' comments; for example, we now show new data from sham-operated groups (new figures 2-5). We believe that because of the constructive criticism of the Reviewers and the Associate Editor, the paper has been considerably improved.

All changes to the text of the manuscript and legends are highlighted in grey. Please see below for our point-by-point responses to the Reviewers' comments.

Sincerely,

Claudia Nold-Petry

Reviewer 1

This manuscript examines the potential of single bolus treatment with a synthetic mu-opioid agonist remifentanil (1ug/kg) to protect against intestinal ischemia-reperfusion injury in C57BL6 mice, as induced by 30 minute occlusion of the superior mesenteric artery.

This builds on a literature base focussed on ischemic pre-conditioning and the role of endogenous and exogenous opioids. The work is justified on the basis that remifentanil has been used in other models and is attractive given its short plasma half-life and hence limited potential to promote/exacerbate surgical ileus.

End-points were measured in plasma (IL-6) and small intestinal tissue (MDA and histologicial scoring) recovered at 60 minutes post-reperfusion. Intestinal segments were stratified into proximal (jejunal) and distal (ileal) sections for analysis. Chi2 and Mann-Whitney tests were used for group comparisons.
The authors demonstrate a reduction in histological grade in the remifentanil group, most notable in the analysis of distal segments. This parallels relative reduction in local measures of MDA and systemic measures of IL-6.

The question posed by the authors is well defined and the methods are appropriate and well described. The data are sound but incomplete.

Major Compulsory Revisions:

1. In terms of optimal scientific method, the lack of control non IRI groups does not allow the reviewer to assess the validity of the scoring system, particularly with regard to mild grades of inflammation in which the characteristic features have potential to resemble preparation artefacts. The authors should grade the intestines of a group of control mice and include this data. These need not necessarily be sham operated.

2. Measurement of the plasma IL-6 levels should be underpinned by measures in baseline control animals.

We thank Reviewer 1 for raising this important issue. Indeed, control non-IRI groups are necessary to validate the scoring system as well as the dataset overall. We have therefore added data in this regard to the revised version of our manuscript. In order to optimize comparability, we decided to invest more work and performed sham operations with saline or remifentanil pretreatment under the exact same conditions as were used for the ischemia-reperfusion (IR) experiments. The new data are included in the revised figures 2-5, with representative histological sections shown in figure 2, histological grading in figure 3, gut tissue MDA in figure 4, and plasma IL-6 in figure 5.

Minor Revisions

3. Discussion of Bcl-2/BAX should make reference to their role in apoptosis

Thank you for pointing this out, we have revised the manuscript accordingly in the Background and Discussion sections.

4. The explanation of a reduced difference between treatment groups in proximal regions as being a consequence of collateral perfusion is not coherent with the observation that injury in saline treated animals is similar proximally and distally. Amend.

The sham-operated groups have provided new, partially unexpected insights into the biology of the intestine in our model. We did not anticipate to find that lipid peroxidation occurs at considerable levels not only in the IR groups, but also in the sham-operated control groups in the jejunum. This is in stark contrast to the ileum, where levels of oxidative stress
were low after sham laparotomy. This observation underscores that Reviewer 1’s point is correct.

Together with the histological results that show reduced effectiveness of remifentanil in the jejunum post-IRI, the new data suggest that there are differences between the jejunum and the ileum in the biological aspects investigated in this study. A literature search revealed that similar observations have been made by other groups. For instance, a study of LPS-induced intestinal injury showed that MDA was increased only in the ileum, not in the jejunum, following injury [1]. Similarly, a study of intestinal IRI using pharmacological conditioning with isoflurane reported protection only in the distal, but not the proximal gut [2]. We have included these references in the revised Discussion. Furthermore, we cannot rule out that the timing of the regulation of lipid peroxidation in the jejunum is different from that in the ileum and that the timepoint we chose is not optimal to show differences that may occur earlier or later. It is unlikely that there is no difference in MDA at any time, as the ischemia-reperfusion-induced injury is not very different between jejunum and ileum (i.e. comparing the sham groups with the IR-saline groups). These aspects have also been addressed in the revised Discussion.

As this issue is rather complicated, we decided to improve clarity for the reader by removing the data on MDA in the jejunum from the revised figure 4. Instead, we now explain these data in the pertaining section of the Results.

Reviewer 2

In this study, authors show that Remifentanil is useful in the management of intestinal ischaemia/reperfusion in mice by assessing histological damage, MDA and IL-6. This is a commendable aim as there is little known on the treatment/preconditioning of this disease with severe consequences. Although the subject matter of the paper is of interest, the main concern is that some of the data sets seem rather preliminary and mechanistic studies are completely absent. Overall, this study does not provide a solid insight into the action of Remifentanil on intestinal ischemia/reperfusion. More studies should be carried out in order to elucidate how remifentanil works in this condition, for instance any affect of the drug on MAPK phosphorylation.

Associate Editor Comment-It will be sufficient for the authors to include a paragraph in a revised version dealing with how the anti-inflammatory pre-conditioning effect of mu-opioid receptor activation is transduced in the cell. This acts on reviewer two's request for more insight into mechanisms in terms of signaling pathways.
We thank Reviewer 2 for the comments regarding our manuscript. We agree that it would be interesting to explore the signal transduction pathways that mediate the beneficial effects of remifentanil preconditioning on the intestine, particularly since this a field where information is not abundant. However, thorough exploration of this aspect is outside the scope of this study. Instead, we have performed an in-depth literature search. In the extensively revised Discussion, we elaborate on the signaling cascades by which remifentanil exerts its protective effects, and explain how this regulation of pathways such as NF-κB, PI-3K/Akt, JNK, p38MAPK and ERK likely explains the findings we present.

We disagree with the notion that the manuscript is preliminary in nature, as well as with the opinion that the mechanistic insights are absent or not solid. Our statistical analyses have shown highly consistent differences between the treatment groups. IL-6 is a well-accepted readout to assess the inflammatory response [3, 4], as is MDA to quantify oxidative stress [5, 6]. Oxidative stress is firmly established as a crucial facilitator of tissue injury caused by ischemia-reperfusion [7, 8]. Therefore, in addition to the observational histology, we have provided sound evidence that 1) remifentanil preconditioning protects the intestine by reducing oxidative stress locally, and 2) that the systemic inflammatory response (with all its potentially deleterious consequences for the intestine and other organs) is inhibited as well. In addition, following the important advice of Reviewer 1, the revised version contains data from sham-operated animals, which yet again considerably strengthen our data. The addition of animals that underwent sham laparotomy has provided us with further evidence that key parameters of IRI are returned to levels that are nearly identical to background. This is now shown in our revised figures 2-5.

References

