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Dear Dr

Jin-Zhou Tang

Science Editor Office,

Baishideng Publishing Group Inc

We would like to thank the editor and the reviewer for their helpful comments and insight regarding our previous submission. We have made the changes discussed below to address the reviewer and Editor concerns.

PEER-REVIEW REPORT

Reviewer's code: 03254146

SPECIFIC COMMENTS TO AUTHORS

I'm pleased to review the paper entitled "P2X7 receptor antagonist recovers ileum myenteric neurons after experimental ulcerative colitis". Present article evaluated the effects of a P2X7 inhibitor, BBG in TNBS-induced ileal enteric neurons decreses and found that all type neurons and GFAP-ir glial cells were decreased in the TNBS group and recovered in the BBG group and that nNOS-ir neurons decreased in the TNBS group and recovered in the BBG group. They suggested that the P2X7 receptor is an important target in therapeutic strategies.

#1 Minor points

1）neuronal nitric oxide synthase is usually abbreviated as "nNOS".

R: We would like to thank the reviewer for his helpful commentaries in the revision. We have included this information in the manuscript. OK

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2) Recently gastrointestinal epithelia responding physiological and pathological stimuli is also focused as ATP origin. Digestion 2020;101:6–11

R: Thank you for this consideration. We have included this information in the manuscript. OK

3) Intestinal movement is regulated by a mechano-sensitive ion channel, TRPV2 and nNOS expressed in inhibitory motor neurons. Please make additional discussion. Journal of Neuroscience 8 December 2010, 30 (49) 16536-16544

R: Thank you for this consideration. We have included this information in the manuscript. OK

4) ChAT positive neurons are divided into excitatory motor neuron and IPAN. Please add data if available, or give comments on these two types of neuron in discussion section.

R: Thank you for this consideration. We agree to the reviewer that this information. We have add this issue in the manuscript. OK

5) They described "In ulcerative colitis, there are changes in enteric nervous system populations [6, 7, 8, 9, 10]."However The paper 9 is an only paper on human ulcerative colitis.

R: Thank you for this consideration. Tthe paragraph is about changes in enteric neurons in ulcerative colitis. Reference [9] provides information of humans. OK

9. Neunlist M, Aubert P, Toquet C, Oreshkova T, Barouk J, Lehur PA, Schemann M, Galmiche JP. Changes in chemical coding of myenteric neurones in ulcerative colitis. *Gut*

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2003; **52**(1): 84-90 [PMID: WOS:000180096600022 DOI: 10.1136/gut.52.1.84]

We decided to include the human term in the paragraph, since the references are in animals, we add the information human in the text. OK

6) Reference 10, "myrenteric"

R: Thank you for this consideration. We have corrected this issue in the manuscript. OK

Manuscript NO: 54064

SPECIFIC COMMENTS TO AUTHORS

This paper performed immnunohistochemistry for P2X7 receptor using ileal tissues of rats received TNBS with or without P2X7 receptor antagonist, BBG. It is interesting that the numbers of myenteric neurons and glial cells in the ileum were decreased in TNBN colitis. However, there are many issues to be addressed.

1. Only one experiment was performed in this study. It seems to be a preliminary examination.

   R We would like to thank the reviewer for his helpful commentaries in the revision.

   I would like to say that We have used for Immunohistochemistry Experiments: 5 animals for Sham animals which were injected with vehicle. 5 animals for BBG (50 mg/kg, Sigma Aldrich, United Kingdom, n=5) or 5 animals for saline was injected 1 hour following TNBS injection (n = 5). For Histological Analysis We used samples of ileum from the sham (n = 3), TNBS (n=3) and BBG (n=3) groups. OK

2. Colonic tissues were not evaluated, so it is uncertain whether changes in DAI

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were due to inflammation in the colon.

R: Thank you for this consideration. We agree to the reviewer that this information. We have add the pictures of distal colon in the manuscript.OK

3. The authors merely evaluated colocalization of P2X7 receptor and NOSn, ChAT, HuC/D or GFAP. It is necessary to demonstrate its significance in TNBS colitis.

R: Thank you for this consideration. We have done counting of colocalization in the 5 animals for Sham animals, 5 animals for BBG and 5 animals for saline and the P2X7 receptor immunoreactivity colocalized 100% with neurons positive for HuC/D, nNOS, ChAT and GFAP in all groups. There was no significant difference.

The methods used was antigen colocalization was determined by analysis of fluorescently labeled preparations. Neurons were identified by immunofluorescence, and the filter was changed to examine the second antigen. Thus, the proportion of neurons labeled by pairs of antigens was determined. The cohort size was 100 neurons, and the data were collected from preparations obtained from five animals. The percentages of double-IR neurons were calculated and expressed as the means ± standard errors (SEM, n = number of preparations). In total, 100 neurons and 100 enteric glial cells from each membrane preparation were analyzed from each of the sham (n = 5), TNBS (n = 5) and BBG (n = 5) groups. OK

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Manuscript NO: 54064

SPECIFIC COMMENTS TO AUTHORS

I'm pleased to re-review the paper entitled "P2X7 receptor antagonist recovers ileum myenteric neurons after experimental ulcerative colitis". Minor points 1） Legend for figure 2 includes "However", which is inappropriate for figure legend. 2) I can not understand the last sentence, "Also, has been described that the expansion of the inflammatory process from the distal neck to the distal ileum". What is noun and what is the "neck" ? 3) "NOSn" is still remaining in Fugure 7.

Minor points

1） Legend for figure 2 includes "However", which is inappropriate for figure legend.

R: We would like to thank the reviewer for his helpful commentaries in the revision. We have corrected this information in the legend for Figure 2.

2) I can not understand the last sentence, "Also, has been described that the expansion of the inflammatory process from the distal neck to the distal ileum". What is noun and what is the "neck" ?

R: We would like to thank the reviewer for his helpful commentaries in the revision. The "distal neck" is wrong word, this issue was corrected.

3) "NOSn" is still remaining in Fugure 7.

R: We would like to thank the reviewer for his helpful commentaries in the revision. This issue was corrected.