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PEER-REVIEW REPORT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 73068

Title: X7 Receptor Blockade Decreases Inflammation, Apoptosis, and Enteric Neuron Loss during Clostridioides difficile Toxin A-induced Ileitis in Mice

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 04427657
Position: Editorial Board
Academic degree: MD
Professional title: Chief Physician, Professor
Reviewer's Country/Territory: China
Author's Country/Territory: Brazil

Manuscript submission date: 2021-11-09

Reviewer chosen by: Xin Liu

Reviewer accepted review: 2022-03-01 01:44

Reviewer performed review: 2022-03-01 04:16

Review time: 2 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [Y] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The study aimed to characterize a specific population of TcdA-affected myenteric neurons and investigate the role of P2X7 in TcdA-induced ileum inflammation, cell death, and enteric nervous system (ENS) changes in mice. And the authors conclude that TcdA induces the upregulation of the P2X7 receptor, which promotes enteric neuron loss, S100B synthesis, tissue damage, inflammation and cell death in the ileum of mice. These findings contribute to future directions in understanding the mechanism involved in intestinal dysfunction reported in patients with pos-CDI. It was an interesting and a good-writing paper. However, I have some comments. 1. The necessity and hypothesis of this study should be mentioned in the Introducion. 2. The grouping was not clear. 3. The magnifications and the conditions of observers in every histological analysis should be described appropriately. 4. Primers of β-actin should be presented in real-time PCR section. 5. The Discussion should be carried out around the data obtained from the study. I noticed that some mechanisms were not clearly elaborated. The authors should reorganize some contents and place their emphases on the mechanisms. 6. The limitations and perspective of this present study should be involved in the Discussion. The clinical significances of this study should be mentioned in the Discussion. 7. The images in Figures 1, 2, 3 and 5 were not clear, and the identifying arrow heads were not given explanations in Figure legends. 8. The documents of “73068-Biostatistics Review Certificate” and “73068-Institutional Review Board Approval Form or Document” were not applicable.

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Peer-review model: Single blind

Reviewer's code: 03538691

Position: Editorial Board

Academic degree: MD

Professional title: Associate Professor, Chief Doctor

Reviewer's Country/Territory: China

Author's Country/Territory: Brazil

Manuscript submission date: 2021-11-09

Reviewer chosen by: Xin Liu

Reviewer accepted review: 2022-02-28 15:09

Reviewer performed review: 2022-03-07 10:54

Review time: 6 Days and 19 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

1【line 154 : Swiss mice (n=5 per group)】【line 591: or PBS alone (Control) (n=4).】【line 593: in 5-6 microscope fields per sample (n=4 subjects per group).】【line 601: challenged mice (n=4 subjects per group).】【line 612: in the ileum myenteric plexus from control and TcdA-challenged mice (n=4).】【line 659: challenged (TcdA, TcdA+A438079) mice (n=4 subjects per】 Why had some experimental groups 5 nude mice? And the other groups had 4 nude mice? The number of animals is different. Can they be compared? 2【line 591-593 : Quantification of the percentage (mean ± s.e.m.) of the P2X7-immunopositive area in ileum from control and TcdA-challenged mice in 5-6 microscope fields per sample (n=4 subjects per group).】 There are only 4 nude mice. Is there sampling error in the test data? Does the test data have normal distribution? Can it be used as mean ± standard deviation to describe?

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Peer-review model: Single blind

Reviewer's code: 02498841
Position: Editor-in-Chief
Academic degree: MD, PhD
Professional title: Dean, Professor
Reviewer's Country/Territory: China
Author's Country/Territory: Brazil

Manuscript submission date: 2021-11-09

Reviewer chosen by: Xin Liu

Reviewer accepted review: 2022-03-04 08:51

Reviewer performed review: 2022-03-13 09:25

Review time: 9 Days

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

It has demonstrated that lack of a functioning P2X7 receptor leads to increased susceptibility to toxoplasmic Ileitis (PLoS One. 2015 Jun 8;10(6):e0129048.), which supports the thesis that P2X7R, a well-documented activator of pro-inflammatory cytokine production, also plays an important role in the regulation of intestinal inflammation. In this paper, authors found that TcdA induced the upregulation of the P2X7 receptor, which promoted enteric neuron loss, S100B synthesis, tissue damage, inflammation and cell death in the ileum of mice, these results are interesting and some innovations. However, there are some concerns as follows: 1. P2X7R has been found to mediate mast cell-dependent intestinal inflammation and inflammation-induced death of enteric neurons via an inflammasome-dependent pathway. P2X7R is also well known as an activator of the inflammasome, a complex of cytosolic proteins that regulates caspase-1 activation and the processing of IL-1β and IL-18 from inactive to active forms. In the present study, caspase-1 activation and IL-18 levels should be examined. In addition, the severity of the ileum damage should be further determined by analysis ileum permeability, not only by histological analysis 2. In the METHODS of abstract, the sentence in Line 57 'To investigate the role of P2X7, BBG (50 mg/kg, i.p.) and A438079 (0.7 μg/mice, i.p.) were injected one hour prior to a TcdA-challenge.' should be revised as 'To investigate the role of P2X7 receptor, Brilliant Blue G (BBG, 50 mg/kg, i.p.), a nonspecific P2X7 receptor antagonist or A438079 (0.7 μg/mice, i.p.), a competitive P2X7 receptor antagonist, were injected one hour prior to a TcdA-challenge.' 3. In introduction, P2X7R (Line 129) should be described before the existing problems in this area (Line 125, However, a knowledge gap remains regarding the population of enteric

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neurons affected by TcdA and the role of the P2X7 receptor in TcdA-induced alterations in enteric neurons and enteric glial cell (EGC)-derived mediators, particularly S100B.) were put forward. 4. In the Methods and Results, levels of IL-1β, IL-6, IL-8 and TNF-alpha should be expressed as pg/mg of tissue, not pg/mL. In the methods, levels of IL-1β, IL-6, IL-8 and TNF-alpha were detected by enzyme-linked immunosorbent assay. However, in the results, levels of IL-1β, IL-6, KC and TNF-alpha were present, the description should be consistent. 5. In Statistical analysis, the histological score should be tested by nonparametric test. 6. In the results, the effects of P2X7 receptor antagonists on nNOS+, Calr+ and ChaT+ neurons should be described in detail. 7. In discussion, the present study demonstrated that P2X7 receptor blockage decreases inflammation, apoptosis and enteric neuron loss during Clostridioides difficile Toxin A-induced ileitis in mice. However, it has demonstrated that lack of a functioning P2X7 receptor leads to increased susceptibility to toxoplasmic Ileitis. The difference should be discussed.

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 73068

Title: X7 Receptor Blockade Decreases Inflammation, Apoptosis, and Enteric Neuron Loss during Clostridioides difficile Toxin A-induced Ileitis in Mice

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Peer-review model: Single blind

Reviewer's code: 04427657

Position: Editorial Board

Academic degree: MD

Professional title: Chief Physician, Professor

Reviewer's Country/Territory: China

Author's Country/Territory: Brazil

Manuscript submission date: 2021-11-09

Reviewer chosen by: Jia-Ru Fan

Reviewer accepted review: 2022-05-12 11:42

Reviewer performed review: 2022-05-12 12:22

Review time: 1 Hour

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [Y] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The manuscript has been greatly improved after revisions.