

Editor-in-Chief of the Journal  
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Dear Editor,

Thank you for allowing us to submit a revised draft of our manuscript titled “Polyethylene glycol 35 ameliorates pancreatic inflammatory response in cerulein-induced acute pancreatitis in rats” to World Journal of Gastroenterology. We appreciate the reviewer and Editorial office comments, which helped us to revise the manuscript. We have been able to incorporate changes to reflect the suggestions provided by them. Our responses to the comments are outlined point-by-point in this cover letter (in blue). As well, we have marked in red the changes within the manuscript.

Thank you very much in advance.

Sincerely yours

Dr. Emma Folch-Puy, PhD  
Experimental Pathology Department, IIBB-CSIC  
c/ Rosellón 161, 7 º 08036 Barcelona, Spain  
Tel (34) 93 363 83 00 ext 357, Fax (34) 93 363 83 01  
E-mail: emma.folch@iibb.csic.es

**POINT-BY-POINT RESPONSES (in blue)**

**REVIEWER #1**

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: The aim of the study was to evaluate the protective effect of PEG 35 on the pancreatic damage associated to cerulein-induced acute

pancreatitis in vivo and in vitro. The experimental results confirmed that PEG 35 could relieve acute pancreatitis, providing a new idea for clinical treatment. In vivo experiments, lipase activity, pancreas wet-dry weight ratio, Lactate dehydrogenase of plasma, real-time qRT-PCR for inflammatory factor expression and WB for pancreatic apoptotic protein expression were detected. PEG 35 was proved to ameliorate acute pancreatitis from multiple perspectives.

We thank the reviewer for this comment and, according with the suggestion, we have now included Hematoxylin and Eosin staining images representative of each experimental group. This analysis has been now incorporated in the revised manuscript as part of Figure 1 (Figure 1C, page 22) and in the various sections of the paper, as follows: in the Abstract section of the paper (page 2), under the new heading “Histopathological Examination” in Material and Methods (page 7-8), under the new heading “PEG35 reduced local pancreatic tissue damage associated with cerulein-induced AP” in the Results section (page 11-12), in the Discussion section (page 14), and in the figure legends of Figure 1. Additionally, sampling and analysis explanation in the “Experimental animals and model of cerulein-induced AP” section of Material and Methods has been changed in other to better clarify the pancreatic tissue samples processing (page 7).

In vitro experiment, AR42J cells were stimulated with three different stimulus (TNF $\alpha$ , staurosporine or cerulein), and the effects of different concentrations of PEG 35 were observed. PEG 35 was shown to reduce inflammatory responses and cell damage in a concentration-dependent manner. If cerulein group can be added to detect apoptosis-related indicators of the cell supernatant or check the apoptosis-related index by Flow Cytometry, it can be further confirmed from in vitro and in vivo experiments that PEG 35 not only relieve inflammation, but also ameliorate apoptosis. General opinion: the author selected a novel topic, no obvious flaw in detection methods, we recommend.

We agree with the reviewer that the measure of another apoptosis-related marker in the *in vitro* cerulein-treated cells would firmly confirm that PEG35 ameliorates apoptosis. We apologize for not being able to perform this assay. Instead, we have performed Lactate dehydrogenase (LDH) activity assay in the cell cultured supernatants from the cells treated with cerulein as in the manuscript we had only shown the LDH activity

data from TNF $\alpha$ -treated cells. The new results from this assay will more accurately reflect the *in vivo* LDH results obtained in cerulein-induced rats. This analysis has been now incorporated in the revised manuscript as part of Figure 4 (Figure 4D, page 26) and in the various sections of the paper, as follows: under the heading “PEG35 reduced inflammation-associated cell death in models of pancreatic damage *in vitro*” in the Results section (page 13), in the Discussion section (page 15), and in the figure legends of Figure 4.

## **EDITORIAL OFFICE’S COMMENTS**

Authors must revise the manuscript according to the Editorial Office’s comments and suggestions, which are listed below:

(1) Science Editor: 1 Scientific quality: The manuscript describes a basic study of the PEG35 protection against cerulein-induced acute pancreatitis. The topic is within the scope of the WJG. (1) Classification: Grade B; (2) Summary of the Peer-Review Report: The aim of the study was to evaluate the protective effect of PEG 35 on the pancreatic damage associated to cerulein-induced acute pancreatitis *in vivo* and *in vitro*. The experimental results confirmed that PEG 35 could relieve acute pancreatitis, providing a new idea for clinical treatment. It would be more perfect if the author could provide the HE staining pathology of the 3 groups.

We thank the reviewer for this comment and, according with the suggestion, we have now included Hematoxylin and Eosin staining images representative of each experimental group. This analysis has been now incorporated in the revised manuscript as part of Figure 1 (Figure 1C, page 22) and in the various sections of the paper, as follows: in the Abstract section of the paper (page 2), under the new heading “Histopathological Examination” in Material and Methods (page 7-8), under the new heading “PEG35 reduced local pancreatic tissue damage associated with cerulein-induced AP” in the Results section (page 11-12), in the Discussion section (page 14), and in the figure legends of Figure 1. Additionally, sampling and analysis explanation in the “Experimental animals and model of cerulein-induced AP” section of Material and Methods has been changed in other to better clarify the pancreatic tissue samples processing (page 7).

The questions raised by the reviewers should be answered; and (3) Format: There are 4 figures. A total of 25 references are cited, including 4 references published in the last 3 years. There are 2 self-citations. 2 Language evaluation: Classification: Grade B. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement, Institutional Animal Care and Use Committee Approval Form, and the ARRIVE guidelines. No humans are involved in the study. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an unsolicited manuscript. The study was supported by Ministerio de Ciencia e Innovación. The topic has not previously been published in the WJG. The corresponding author has published 1 article in the BPG. This manuscript is the resubmission of Manuscript No. 53667. 5 Issues raised: (1) I found the authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);

[The approved grant application document has been uploaded.](#)

(2) I found the authors did not provide the original figures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

[Thank you for this observation. We have now provided the original figures documents prepared using powerPoint.](#)

(3) I found the authors did not write the “article highlight” section. Please write the “article highlights” section at the end of the main text; and

[Taking into account the Editorial office suggestion, we have now written the “Article highlight” at the end of the main text \(in red; page 16-17\).](#)

(4) please don't include any \*, #, †, §, ‡, ¥, @....in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters. Statistical significance is expressed as aP < 0.05, bP < 0.01 (P > 0.05 usually does not need to be denoted). If there are other series of P values, cP < 0.05 and dP < 0.01 are used, and a third series of P values is expressed as eP < 0.05 and fP < 0.01.

As suggested by the Editorial office, we have replaced superscript symbols “\*” and “+” for superscript letters “a” and “b” for statistical significance along the revised manuscript. The changes have been reflected in the figures and figure legends of each figure.

6 Re-Review: Not required. 7 Recommendation: Conditionally accepted.

We would also like to point out that we have corrected, in the revised manuscript, some missing information we have found within the “Material Methods” section of the manuscript:

- We forgot to include that LDH activity was also measured in the cell culture supernatants. This information has now been included (in red) in the “Lactate dehydrogenase activity” subsection (page 9 of the revised manuscript).
- We failed to include the use of IL10 and iNOS primers for real-time qRT-PCR in the “Real-time qRT-PCR” subsection. This information has now been included (in red) in page 10 of the revised manuscript.

As well, we have followed the steps 1 to 8 from the received e-mail from the BPG editorial office (on august 8th) to meet the requirements for final acceptance and publication.

Thank you very much in advance.

Sincerely yours

Dr. Emma Folch-Puy, PhD  
Experimental Pathology Department, IIBB-CSIC  
c/ Rosellón 161, 7 º 08036 Barcelona, Spain  
Tel (34) 93 363 83 00 ext 357, Fax (34) 93 363 83 01  
E-mail: emma.folch@iibb.csic.es