Format for ANSWERING REVIEWERS

October 12, 2017

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 36076-Answering reviewers.doc).

Title: Emodin and Baicalein inhibit sodium taurocholate-induced vacuole formation in pancreatic acinar cells

Author: Jun Li, Rui Zhou, Beibei Bie, Na Huang, Ying Guo, Haiyan Chen, Mengjiao Shi, Jun Yang, Jian Zhang, Zongfang Li

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 36076

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Why were there only male rats used?

Answer: Because of the diversity of hormone levels of female rats, we chose the male rats for animal experiment.

(2) Have the authors compared the effect of CEB to the effects of each component by itself?

Answer: In our previously study, we have performed animal experiment to compare the effect of CEB to the effects of each component. The result showed that CEB displayed a better therapeutic

effect than each component. Therefore, we perform this study to investigate the mechanisms of combined use of emodin and baicalein at cellular and organism levels with SAP.

(3) How were the doses chosen?

Answer: In our previously study, we used weighted modification method and performed animal experiment to investigate the dosage optimization of CEB. The optimal dose of CEB (3.2 mg/kg dose of Emodin combined with 7 mg/kg dose of Baicalein) was chose as the middle dose in this article and the relevant manuscript have accepted by Chinese Journal of Integrated Traditional And Western Mdeicine (Manuscript No. 2017-1448).

(4) How was the 12hr time point chosen? Did the authors look at other time points?

Answer: Retrograde injection of 5% sodium taurocholate into the common biliopancreatic duct is a generally method used for SAP model induction in experiment research for a long time. Because of the high mortality from 15h after SAP induction, we chose 12h as the time point.

(5) What are the side effects of CEB? In humans what are the data for its use?

Answer: By time, we have investigated the effects of CEB at cellular and animal levels and have no data for its use in humans. At cellular level, CEB showed no side effect on the normal pancriatic acinar cells at either low or high concentrations in this study. At animal level, CEB showed good therapeutic effect but no side effect on SAP rats. This preclinical research provides potential clinical benifits and needs to be lucubrate in the future.

(6) Could the authors include in the discussion a paragraph about the limitations of the study?

Answer: We would like to thank the reviewer for the comments about the limitations of the study. Some statement about the limitations of the

study has been added in the end of the text.

(7) These compounds will undoubtedly suffer in the way many others have suffered. They will work well when given in close proximity to the stimulus for the attack but in clinical practice this will not be possible. The authors however make no promises that they will and recognise that further study is required.

Answer: We would like to thank the reviewer for the comments about the clinical practice of CEB. By time, the study on CEB remains at the preclinical level and the data for its use in humans are lacking, which urge us to make more effort to do more further study. Some relevant statement has been added in the end of the text.

(8) The manuscript adheres to the Journal's standards and scope. Potentially, the paper is interesting to WJG readership and relevant to the field. However, many pieces of information are lacking, mainly in the description of procedures and results (including text, figure legends and abstract). These aspects should be revised in order to be acceptable for publication in WJG.

Answer: We would like to thank the reviewer for the comments about the lacking information. The aspects has been added in the text.

(9) The length of the title and running title is fine, but they both focus only on a very specific aspect of the work (vacuole formation).

Answer: The vacuolization is a lable of cell injury and reflects the degree of cell injury, so we focus on vacuole formation in the title.

(10) The abstract conforms to the preparation guidelines outlined in the Writing Requirements of Basic Study. However, it should be more informative.

Answer: More information has been added in the text.

(11) Language evaluation: Minor language polishing

Answer: English grammar and construction were edited by a professional English language editing companies.

(12) Abstract

Methods: brief description of procedures (pretreatment-treatment) should be included, both for rats and isolated cells. It should be mentioned that the cell study was made in cells prepared from healthy rats.

Answer: The brief description of procedures has been added in the abstract.

(13) Abstract

Results: Make clear that (2.07 ± 1.20 vs 6.84 ± 1.13, P<0.05) refers to histopathology score, not amylase. In the results section of the abstract, numerical results for some parameters are included.

In these cases, CEB doses or concentrations should be indicated.

Answer: The numerical results have been added in the text.

(14) Materials and Methods

General: Centrifugation conditions would be better described if expressed in units of gravity (times gravity or × g) rather than revolutions per minute, rpm.

Answer: The centrifugation conditions has been corrected in the text.

(15) Materials and Methods

First paragraph in the Materials and Methods section may be entitled Animals instead of Materials (no materials are mentioned or described).

Answer: The title has been replaced with Animals.

(16) Materials and Methods

Therapeutic effects of CEB.

- Line: 5: low-dose instead of high dose; line 8: high-dose instead of low-dose (names "high" and "low" do not correspond with the doses).

- Please, specify route of administration of CEB (intravenous??) and when it was administered after taurocholate (immediately??)

Answer: The doses have been corrected and the route of administration

of CEB has been added in the text.

(17) Materials and Methods

Isolation of rat pancreatic acinar cells

- The title of this subsection should be "Isolation of pancreatic acinar cells from healthy rats"

- In their Pancreapedia article (ref 18), Williams et al mention many different digestion enzymes to be used for the isolation procedures. Please, give details of the collagenase used in this study (manufacturer, type and concentration/activity used for the digestion). Include also details for the trypsin inhibitor (manufacturer, concentration/activity used).

Answer: The title of this subsection has been corrected and the details of the collagenase and trypsin inhibitor have been added in the text.

(18) Materials and Methods

Cell vitality assay.

- MTT assay. If a commercial kit was used, please give details of the name and manufacturer. If it was not a kit, please include a literature reference.

- This subsection (as a whole) is very confusing. When comparing the text of this paragraph with the corresponding one in the Results section and the corresponding figure (Figure 2), I have come to determine that 3 diffrerent experiments have been done: 1) NaTC alone (different concentrations and different incubation times); 2) NaTC 8 mM for 30 min (?) after 10-min pretreatment with different concentrations of CEB; 3) CEB alone (time?) at different concentrations. Still, I am not sure that this is what the authors really performed…The protocols used (design of the different experiments) are scarcely described (substances involved, pretreatment or not, times, concentrations, etc). The subsection may be completely rewritten.

Answer: The literature reference for MTT assay has been updated. There

were three diffrerent experiments in this subsection as the reviewer said and we have revised this subsection. 1) NaTC alone (different concentrations and different incubation times) to screen a proper concentrationof sodium taurocholate (8 mM) for next cell experiments; 2) NaTC 8 mM for 30 min after 10-min pretreatment with different concentrations of CEB; 3) CEB alone for 30 min at different concentrations to assess the reliability of CEB at cellular level.

(19) Materials and Methods

Cell ultrastructural observation. I feel curious about the reason why in the cell viability experiments, after the 10-min pretreatment with CEB, the authors used 8 mM NaTC/30 min as a damaging factor, whereas in the cell ultrastructural study, the damaging factor was 1 mM NaTC/1 min. Maybe the readers would thank some explanatory note about this.

Answer: It has been reported that cytosolic Ca^{2+} overload is related to acinar cell injury and the Ca^{2+} signal changed in a few minutes. So we chose 1 mM NaTC/1 min as a damaging factor instead of 8 mM NaTC/30 min in the cell ultrastructural study, intracellular free Ca^{2+} study and IP_3R expression study.

(20) Materials and Methods

Intracellular Ca^{2+} measurement. Please indicate manufacturer for the fluo-3 AM probe.

Answer: The manufacturer for the fluo-3 AM probe has been added in the text.

(21) Materials and Methods

Quantified IP_3R expression. Please, indicate manufacturer for the RNA and protein extraction kit, and provide some literature reference for the RT-PCR and Western blotting protocol.

Answer: The manufacturer for the RNA and protein extraction kit have been added and the literature reference for the RT-PCR and Western

blotting protocol have been updated.

(22) Results

Serum amylase and inflammatory response. CEB decreased serum IL-6 in SAP rats EXCEPT AT THE LOW DOSE. This should be made clear.

Answer: We have revised the results in the text.

(23) Results

Cell viability. Lines 7-8 of this page should read: "The pre-treatment with CEB could increase the vitality in cells treated with 8 mM NaTC, and showed dose-

dependent protective effects at …" and then (following lines): "Moreover, CEB alone had no adverse effect on the normal cells at either….)

Answer: We would like to thank the reviewer for the good advise and we have revised this subsection.

(24) Results

Vacuoles.

- Line 9 from the bottom of the page: indicate NaTC concentration. ("after one-minute with ….. mM sodium taurocholate"

- Line 4 from the bottom of the page: indicate CEB concentration and pretreatment time.

Answer: We have added thess details in this subsection.

(25) Discussion

1. Last page of the text, line 4 from the top of the page: "We used pancreatic acinar cells prepared from healthy rats that were induced by sodium taurocholate as an …"

Answer: We would like to thank the reviewer for the good advise and we have revised this subsection.

(26) Figures and figure legends

General: The figure legends should help making the figure comprehensible without reference to the text. In this sense, I think

that the authors should give a brief description of the experimental groups/treatments/pretreatments, including times, concentrations, etc).

Answer: We would like to thank the reviewer for the good advise and we have added these details.

(27) Figures and figure legends

1. Figure 2.iii: an explanatory note on the reason why vitality results of the experiments with CEB alone (Fig 2.iii) are nor expressed in the same way as in the previous experiments (Figures 2.i and 2.ii) may be appreciated by the readers.

Answer: We have revised this subsection.

(28) Figures and figure legends

2. Figure 2 legend (ii): "CEB pretreatment increased (instead of decreased???) vitality in cells treated with 8 mM sodium taurocholate and showed dose-dependent protective effect…"

Answer: We would like to thank the reviewer for the elaborative revise and we have modified it in the text.

(29) Figures and figure legends

3. Figure 3i B and C: According to the legend, the sharp increase in cytosolic calcium ofin the first 2 seconds is due to addition of NaTCI. Have the authors explored the possibility that this is some artefact???

Answer: It has been verified in many articles that bile acids (include sodium taurocholate, taurolithocholic acid 3-sulfate, et al) can initiate pathological Ca^{2+} elevation, serves as a key contributor to the initiation of cell injury, which is crucial in the development of pancreatitis. The results of intracellular Ca^{2+} concentration in our study were actual.

(30) Figures and figure legends

4. Figure 4i: title on Y-axis: "mRNA relative expression (% of control)"

Answer: We have revised the title of Y-axis in the text.

(31) Figures and figure legends

5. Figure 4ii: Have the authors quantified the IP_3R protein bands and

normalized to respective β-actin control?? A bar chart with these data (perhaps in addition to the image) should be more illustrative and informative.

Answer: The results is based on the quantification of IP_3R protein bands and normalization to respective β-actin control. We would like to thank the reviewer for the good advise and we have added the bar chart in the text.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Zongfang Li, MD, PhD
National Local Joint Engineering Research
Center of Biodiagnostics and Biotherapy,
Department of General Surgery, the Second
Affiliated Hospital of Xi'an Jiaotong
University College of Medicine,
157 West 5th Road, Xi'an 710004,
Shaanxi Province, PR. China,
Telephone: +86-29-87678002,
Fax: +86-29-87678634,
E-mail: lzf2568@ xjtu.edu.cn