

March 25, 2014

Dear editor,

Please find enclosed the edited manuscript in Word format (file name: 9316-review.doc).

Title: Esophageal *H. pylori* colonization aggravates esophagitis and promotes the development of Barrett's esophagus and esophageal adenocarcinoma

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Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO: 9316

Thanks a lot for having reviewed our manuscript and sending us the reviewers' reports on our manuscript (Manuscript ID: 9316). Particularly, we would like to thank the reviewers for their valuable comments and criticisms. We also had been extensively revised according to the comments and criticisms.

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 reviewers

Response to Reviewer 00058348:

Question 1: How many mice were initially planned to be used in each of the four randomized groups, based on your previous study? Based on your description, 21 died 6-8 weeks after operation. What was the distribution among the four groups?

Answer: In our previous study, the mortality of rats that underwent EDA was 40-50%, and the prevalence of esophageal *H.pylori* colonization in rats that underwent EDA was 64.3% [*Helicobacter* 2011; 16(1): 66-77.] Therefore, we assigned more rats in the EDA group and EDA with *H. pylori* infection group. Totally, 65 rats were included in this experiment: 8 rats in the pseudo-operation group, 8 rats in the pseudo-operation with *H. pylori*

infection group, 15 rats in the EDA group, 34 rats in the EDA with *H. pylori* infection group. 21 rats of EDA died within 8 weeks after operation: 6 rats in EDA group, 15 rats in EDA with *H. pylori* infection group. No rats died in the pseudo-operation group and pseudo-operation with *H. pylori* infection group. The reasons of dying were complications associated with EDA. Therefore, the died rats was not included in the final analysis. No rats died between 8 and 36 weeks after the operation. This is clearly stated in the methods and the results on page 5, 6 and 10. The repeating contents on page 10 have been deleted.

Question 2: Please describe the gender of the animals used in the experiments.

Answer: All rats were male. This has been stated on page 5.

Question 3: Please describe how the “pseudo-operation” was performed.

Answer: In the two pseudo-operation groups, the abdomen of rats was cut open; the bowels were flipped, and then sutured. This was added in the 12th line on page 16.

Question 4: How many CFUs of *H. pylori* in total were inoculated each time? Was it once daily?

Answer: The concentration of suspension of *H. pylori* was 1×10^8 CFU/mL. 1mL were inoculated every other day, the inoculation was performed for three times totally. It had been written in the section “*H. pylori* culture and inoculation” in the MATERIALS AND METHODS on page 9.

Question 5: I assume that by “both RUT and anti-*H. pylori* antibody positive” only refers to “gastric colonization”. Please clarify.

Answer: The definition of *H. pylori* infection was Warthin-Starry silver staining positive, or both RUT and anti-*H. pylori* antibody positive. This was the same as for the colonization of *H. pylori* both in the stomach and in the esophagus. Samples from the stomach and the lower esophagus were assayed by Warthin-Starry staining and RUT. This was clarified on page 9 line 7.

Question 6: References should be given for the definitions of BE and EAC

Answer: The references have been added on page 7.

Question 7: Please describe clearly the status of *H. pylori* colonization for all the four groups; I am wondering why there was no *H. pylori* colonization in the pseudo-operation with *H. pylori* infection group, even in the stomach if this was the case.

Answer: Only gastric *H. pylori* colonization was found in the pseudo-operation with *H. pylori* infection group. The precondition for esophageal *H. pylori* colonization is the reflux of gastric content and the replacement of columnar epithelium in the lower esophagus. There was no reflux and columnar epithelium replacement in the pseudo-operation with *H. pylori* inoculation group. The status of *H. pylori* colonization for all the four groups had been written in lines 3-6, the section of “general observation”

Question 8: The names of subgroups for the EDA with *H. pylori* infection group appear misleading, which may be modified as “EDA with concomitant esophageal colonization” and “EDA with only gastric colonization”.

Answer: The names of the subgroups in the manuscript have been revised according to the reviewer’s suggestion.

Question 9: What statistical methods were used for numerical parametric data (e.g. ANOVA, or t-test)? χ^2 should be χ^2 .

Answer: The distribution of the numerical values of the relative mRNA expression was not normal. IHC scores were ranked data. Therefore, we employed the non-parametric test to analyze these data. No “ χ^2 ” was used, we wrote χ^2 in the manuscript.

Question 10: The Results section should be made more concise by not repeating the Introduction and Methods, and not interpreting the results.

Answer: Thank you for the advice. We have deleted the repeating parts and make the results more concisely.

Question 11: The conclusion should be firmly drawn based on the major findings obtained from the present study and consistent between the abstract and main text.

Answer: The conclusion has been revised according to the reviewer's suggestion.

Question 12: Modifications should be made for some sentences and terms.

Answer: The manuscript has been edited by professional English language editing company. The certificate letter has been provided. See attachment.

Response to Reviewer 02504712:

Question 1: The authors mention that the severity of oesophageal injury was decreased in rats of EDA with only gastric H-pylori colonisation compared with rats in the EDA group. This is not statistically significant and the authors may wish to revise this sentence.

Answer: As shown in figure 3, there were significant reduction in the scores of esophageal mucosa injury in rats of EDA with only gastric *H. pylori* colonization than in rats of EDA group ($P < 0.05$).

Question 2: The authors mention that "studies have indicated that H-pylori can colonise the gastric type epithelium of the lower oesophagus...to the rest of the sentence". The authors have not really proven this point in this particular study and they need to make this clear by adding "in a previous study" and mention the reference.

Answer: We revise the sentence by "Previous studies have indicated", and add the reference in the second paragraph of page 13.

Question 3: The authors mention, "in our animal model, chronic severe inflammation caused by reflux and H-pylori induced strong oxidative stress and DNA damage". The authors have not illustrated that oxidative stress is indeed what has happened in this model. Either an explanation should be provided or this sentence should be revised.

Answer: This sentence "in our animal model, chronic severe inflammation caused by reflux and *H. pylori* induced strong oxidative stress and DNA damage" had been deleted.

Question 4: The authors have used H-pylori strain SS1. This is a particularly

virulent strain and the authors may need to add a justification for the use of this strain and its effect on this particular model.

Answer: *H. pylori* SS1 is a mouse adapted strain and has a strong ability for colonization. It is commonly used to establish a standard rat model of *H. pylori* infection. This is explained in the discussion in the 1st paragraph on page 14.

Response to Reviewer 00227577:

Question 1: GERD causes esophageal inflammation. If bacterial infection is involved, of course the situation can go worse. Therefore, if you aim to assess the contribution of *H. pylori* in particular to GERD-to-EAC development, another type of bacteria (e.g. *E. coli*) needs to be used as a control, to rule out the general effect of bacterial infection.

Answer: Previous study showed that in a rat model of duodenal reflux caused by esophagojejunostomy, bacterial overgrowth in the rat jejunum was a proposed mechanism of carcinogenesis [1]. The intestinal microflora contains *Lactobacillus*, *Bacteroides*, *Enterococcus*, *E. coli*, etc. We eliminated the possible influence of other bacteria by assign a group of rats that underwent EDA only as control.

Question 2: Bax and Bcl-2 are not always involved in apoptosis. They are the gate keepers for mitochondrial permeability. Therefore, if the apoptosis was triggered through something other than mitochondrial pathway, BAX and Bcl-2 ratio may not be affected. Therefore, examination of BAX and Bcl-2 is not rationalized.

Answer: Previous study has indicated that *H. pylori* induce the apoptosis via mitochondrial pathway [2]. *H. pylori* VacA modulates the permeability of mitochondrial membrane by a transmembrane potential dependent manner, which ultimately resulting in cytochrome C releases. We have added the explanation and reference on page 15.

Question 3: The result section needs some rationalization. For each experiment

that you did, a rationale needs to be presented to let readers know why you chose to do that experiment.

Answer: We have added some sentences in the corresponding paragraph in the results section to explain the rationality of the experiments.

3 References and typesetting were corrected.

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

Sincerely yours,

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References:

- 1 Fein M, Fuchs KH, DeMeester TR, Peters JH, Wittmann D, Weig M. Evaluation of the intestinal microflora in the rat model for esophageal adenocarcinoma. *Dis Esophagus* 2000 2000-01-20; 13(1): 39-43.
- 2 Calvino-Fernandez M, Benito-Martinez S, Parra-Cid T. Oxidative stress by *Helicobacter pylori* causes apoptosis through mitochondrial pathway in gastric epithelial cells. *Apoptosis* 2008 2008-10-01; 13(10): 1267-1280.