

ANSWERING REVIEWERS



April 15, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 16399-review.doc).

Title: Potential Protective Effects of *Clostridium butyricum* on Experimental Gastric Ulcers in Mice

Author: Fangyan Wang, Jiaming Liu, Haihua Luo, Aihua Liu, Yong Jiang

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 16399

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) **Reviewer 1**

In my opinion this experimental paper is very well designed, is clearly written and the discussion is rich. The tables and figures illustrate very well the obtained results and the conclusions are clear. Only in fig. 7 I detected a small mistake. You must change *H. polyiri* by *H. pylori*. That's all!

Answer:

Thanks for the good evaluation to my paper. I have recorrected the spelling in the revised version.

(2) **Reviewer 2**

(A) *This manuscript is of low scientific importance, because authors have used a pretreatment with mixture containing C. butyricum to "treat" ulcer induced by different methods.*

(B) *The conclusions are not supported by the presented data. All the results can be a consequence of a preventive and not a curative effect. Thus, it has little to do with real situations of ulcer.*

Answer:

(A) This expert questioned scientific importance of our paper because of pretreatment with mixture. We have considered this and well designed the experiment. In this study, we pretreated with *C. butyricum* free culture media in the sham control group as control and did not find any protective effects, which can clearly show the effect of *C. butyricum*.

(B) My paper is named "Potential protective effects of *Clostridium butyricum* on experimental gastric ulcers in mice", we did not emphasized the treatment effect. So I think there are some misunderstandings between us. In fact, we are investigating the curative effects on a chronic gastric ulcer model induced by the local injection of acetic acid, as you suggested.

Second, (A) the ethical concerns are serious, because instead of using several models of ulcer induction it would be much more apropos to use one model and explore whether administration of C. butyricum after ulcer induction (and not before) would protect or not the ulcer installation. (B) The conclusions and schemes about the effects of C. butyricum are mere speculation, because the presence of C. butyricum might have a antiulcer effect by the simple fact that a "non-specific" bacterium was administered previous to the ulcer induction.

Answer:

The second question is still based on the therapeutic effects. We have talked about that above.

(A) The ethical inspection file was submitted and has been verified (Permit number: wydw2012-0109). In the study of gastric ulcer, the models of acute gastric ulcers we used in the paper are classical to screen and evaluate drugs for gastric ulcer prevention. As you advised, we planned to investigate the therapeutic effect of C. butyricum on the chronic gastric ulcer model induce by local injection of acetic acid in stomach wall. (B) This paper used three models to replicate gastric ulcer by different causes in human and well proofed the protective effect of C. butyricum on gastric ulcer by the results. As we analyzed in the paper, C. butyricum possesses two advantages to prevent gastric ulcer. The first one is that C. butyricum can colonize in stomach and inhibit H. pylori. The second is that butyrate, the main metabolite of C. butyricum, which can protect intestinal musoa and has been used in clinic for colitis patients. Our another paper (under review) showed butyrate prominently prevents gastric ulcer induced by ethanol. So, it seems that C. butyricum is not so "non-specific" for pretreatment to prevent gastric ulcer.

Third the formulation or pharmaceutical preparation used is questionable. Is it possible to "administrate" bacteria in culture medium? I mean is it possible to have stable and alive C. butyricum at the concentration administered for an instantaneous ingestion by patients?

Answer:

The method of C. butyricum administration referred the published paper ^[1] is conventional way to administrate probiotics in animal study. Here, we emphasized that C. butyricum with the character of acid fast can live in stomach. So, we did not worry about the problem you mentioned and the results also showed that our C. butyricum pretreatment is effective.

(3) Reviewer 3

The number of mice is so high regarding ethical issue. Please add this as limitation.

Answer:

Stomach of mice is small and thin, the total stomach is less than 100mg, while in order to show the effect of C. butyricum exactly, we detected several parameters on three different GU models. So, the number of mice is high. We described ethical rules in our paper. Our Permit number is wydw2012-0109. Thanks for your advise.

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] Takahashi M, Taguchi H, Yamaguchi H, Osaki T, Kamiya S. Studies of the effect of *Clostridium butyricum* on *Helicobacter pylori* in several test models including gnotobiotic mice. *J Med Microbiol.* 2000. 49(7): 635-42.