

September 10th, 2019

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 50517-Manuscript (revise).doc).

Name of journal: World Journal of Gastroenterology

Manuscript NO: 50517

Title: Prebiotic UG1601 mitigates constipation-related events in association with gut microbiota: A randomized placebo-controlled intervention study

Author: Jae Ryang Chu, Saem-Yi Kang, Sung-Eun Kim, Sol-Ji Lee, Young-Chul Lee, Mi-Kyung Sung

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

1) Reviewer (Code: 03202632)

In this manuscript Prof. Sung and his colleagues stated that prebiotic UG1601 supplementation might contribute to alleviation constipation symptoms and endotoxemia by conducting a RCT. They showed serum CD 14 and LPS concentrations were significantly decreased after prebiotic usage. They also compared some abundance of bacteria which may produce SCFA or other metabolites related to constipation.

<Major comments>

1. The authors based all the mechanism that prebiotics affect constipation on the change of SCFA level. However, in this study, no significant change of SCFA was observed between the two groups after prebiotic usage. So the discussion and conclusion are not convincing. I assume that the improvement of constipation symptoms and biomarkers were caused by other reasons the authors have not defined.

>> We fully agree with the reviewer's concern and this is a fair question. Since we did not perform further studies for other possible mechanistic explanations, speculations based on related literatures are provided in the discussion (page 19, lines 376-384).

2. The authors should also describe the stool collection method which is important and special in patients with constipation.

>> We added the detailed information on stool collection method in our manuscript (page 9, line 144-146). The hard stools were broken into small pieces before obtaining the sample.

3. The definition of “responders” and “non-responders” should be given in the method section in detail with reasons or reference.

>> We mentioned the definition of “responders” and “non-responders” in the Method section (page 11-12, line 213-218).

4. In this article, various indicators were examined by the authors. But please explain the reason why you choose them. For example, why CD14 was examined rather than other factors?

>> We described why we chose those indicators in the Method section (page 9, line 149; page 10, line 156-157; page 10, line 164-167; page 10, line 173-174).

5. Similarly, could *Bifidobacterium longum* etc. represent the abundance of acetate-producing bacteria?

>> *Bifidobacterium* spp. is well known for acetate-producing bacteria by fermentation (Fukuda S et al., (2011). *Nature* 469, 543–547; Bindels LB et al., (2015). *Nat Rev Gastroenterol Hepatol.* 12, 303–310). The proportion of *Bifidobacterium* spp. in human gut shows 2-14% and this composition is maintained stably throughout adulthood (Arboleya S et al., (2016). *Front Microbiol.* 19(7) 1204). Especially, it has been known that *B. adolescentis* and *B. catenulatum* are predominant *Bifidobacterium* spp. in adults and *B. longum* is widely spread in all age groups (Kumiko K et al., (2017). *Curr Microbiol.* 74(8). 987-995). Thus, we measured the relative abundance of these three representative acetate-producing bacteria in our study.

6. In general: all of the figure legends need to be much more detailed, important explanations and descriptions are missing.

>> We revised figure legends as suggested (page 32-36).

<Minor comments>

7. The authors should explain why and how “mild constipation” patients (Line 323) was defined.

>> In the present study, we referred to ROME III criteria, which are used for constipation diagnosis (Gastroenterol Rep. 2007 Winter;1(2):56-65). Necessary explanations are provided in the Materials and Methods section (page 8, line 100-104).

8. The methods lack a proper description of how the sample size was decided?

> We described the method of sample size determination in the revised manuscript (page 9, line 127-128).

9. The name of bacteria need to be reported formally.

>> We revised the bacterial names in the manuscript in appropriate manner.

10. P values need to be reported. e.g. Page 14 Line 228

>> P vales were inserted where significant differences are detected.

2) Reviewer (Code: 02445670)

This article investigates the mitigating effect of prebiotic on constipation in association with gut microbiota. Along with the effect of probiotic consumption on constipation, prebiotic consumption is a newer approach to emerge similar or even higher symptoms. The topic and title is interesting with practical merits. The study design is appropriate and a comprehensive set of parameters are measured. I suggest acceptance.

Just, authors must re-check the bacterial names form the stylistic point of view (being italic or non-italic, if the first letter of genera must be written small-e.g., bifidobacteria-, and writing the name of genera in abbreviation-first letter in Capital- after the first emergence in the text instead of writing it in complete).

>> As answered above, we revised the names of bacteria in appropriate manner.

3) Reviewer (Code: 05125469)

In the present manuscript, Prof Sung and co-researcher has explained the importance of prebiotic

UG1601 administration leads to amelioration of constipation related events in adult mild constipation subject. The title and abstract are catchy and well explained about the subject matter.

1. Remove the Firmicutes from the Keywords as it is also coming under the gut microbiota.

>> We removed the “Firmicutes” from the Keywords.

2. The statistical analysis and experimental methods are also well designed. The background section need little modify giving more emphases on the present status and significance of this randomized control trial study.

>> We added more explanations in the Introduction section (page 6, line 87-92).

3. This human trial study has been well executed and lot of factors like (serum cluster of differentiation (CD) 14, lipopolysaccharide (LPS) concentrations, fecal SCFAs Concentration, gut microbiota flora) has been evaluated and showing its associated with constipation. But the clinical diagnostic factor like Bloating & cramping, Hematochezia, increase serum calcium levels and decrease in serum potassium and magnesium levels (Metabolic causes) are some critical factor must included in the discussion part to provide clear cut idea on constipation diagnosis that would be helpful to the practitioner.

>> We appreciate for the reviewer’s very constructive suggestions. This information is inserted in the Discussion section (page 21, line 443-446).

4. If history (depression, dietary type, calcium and/or Iron supplementation, opioids consumption, or any other factor responsible for mild constipation) of this randomized controlled trial subjects are available that must be provided to give an idea about occurrence of constipation.

>> We considered diet, dietary supplements and antibiotic use as important factors possibly affecting constipation symptoms. Also, subjects who has or diagnosed with other diseases are excluded. These are described in the Method section (page 8, line 100-108). Unfortunately, we did not include psychological factors or opioids use as variables, which need to be considered in future studies.

5. And how to distinguish between a mild- and chronic-constipation.

>> We inserted explanations in the revised manuscript (page 8, line 100-104).

6. The author has explained very well about the association of gut microbiota and SCFAs with constipation. In the discussion part author must mention the limitation of the study just above the conclusion portion.

>> We revised the manuscript accordingly (page 20-21, line 430-442).

7. In the conclusion portion mention the significance of the study properly how butyrate producing bacteria contribute to the improvement of the symptom score and endotoxemia, which will provide ideas to the scientific community for further future study.

>> We added this explanation in the Discussion section as suggested (page 21, line 449-452).

8. Re-check the writing style of scientific names both in the manuscript text as well as in figures.

>> We re-check the wiring style in the manuscript and modified accordingly.