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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 39929

Title: VSL#3 Alleviates Ulcerative Colitis Carcinogenesis in Mice

Reviewer's code: 02519674

Reviewer's country: Slovakia

Science editor: Xue-Jiao Wang

Date sent for review: 2018-05-28

Date reviewed: 2018-05-30

Review time: 2 Days

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	[ ] Accept (High priority)	Peer-Review:
[ ] Grade B: Very good	[ ] Grade B: Minor language polishing	[ ] Accept (General priority)	[Y] Anonymous
[Y] Grade C: Good	[Y] Grade C: A great deal of language polishing	[ ] Minor revision	[ ] Onymous
[ ] Grade D: Fair	[ ] Grade D: Rejection	[Y] Major revision	Peer-reviewer's expertise on the topic of the manuscript:
[ ] Grade E: Do not publish		[ ] Rejection	[Y] Advanced
			[ ] General
			[ ] No expertise
			Conflicts-of-Interest:
			[ ] Yes
			[Y] No

SPECIFIC COMMENTS TO AUTHORS

The overall scientific quality and importance of the manuscript is good, but not outstanding. I have several comments.

1. Figure 1: probably some symbols missing. All treated groups look the same. it is not clear when exactly the treatment was applied and how.

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Answer： I have already corrected it.

2. In the methods section "lavage" and "gavage" are being used interchangeably. Which one is the right means of administration?

Answer： I have already change all “lavage” into “gavage”.

3. The manuscript need extensive language editing. There are numerous inconsistencies and wrong scientific expressions (e.g. plasmid nuclear ratio) throughout the text.

Answer： Thank you for your advice, I have already reedited the expressions.

4.In the Results the authors claim that "As shown in figure 2, compared with the control mice, body weight loss was significant in mice treated with AOM/DSS..." Figure 2, however, does not show any significant difference between groups. Also, what are "other colitis symptoms"? Data on stool consistency are missing.

Answer： In Figure 2，the first line represents the control group, and the remaining four lines indicate the AOM/DSS treated groups (no matter what treatment was applied), it is clear that after week 2, the body weight in AOM/DSS treated groups began to decrease, while the body weight in control group still climb up. Maybe I should correct my expressions as “after day 10 of DSS administration”, which is more clear. It’s difficult to assess stool consistency by precise data, it’s just a description by observation. And “the other colitis symptoms” may be not accurate, so I have changed my expression as “accompanied by colitis symptoms, such as loose and bloody stool and dim body hair, fatigue, and less movement”.

5.What software was used for analysis of microbiota and for the visualization of the results (heatmap)? The authors conclude an increase or a decrease in some microbial

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species after treatment, but this is not obvious from the data/figure. The heatmap only shows one time point, so it is not possible to conclude any increase or decrease within the respective groups in time. It shows the difference between the groups after 12 weeks. So all the differences (I would prefer the terms higher or lower abundance) are strictly compared to the control group at 12 weeks. Similarly in Discussion: "For mucosal microbiota, we found that Bifidobacterium increased in mucosa after VSL#3 supplementation". The authors did not show any change in the group treated by VSL#3. They only showed differences between groups at 12 weeks.

Answer： We use software qiime (version v.1.8) to analyse alpha diversity, and software mothur to analyse the microbiota differences between groups by metastats. And we use software R, Vegan package for the visualization of the heatmap. I agree with you that heatmap can not show the differences very clearly, so I have changed it into table 4 and 5 which contains accurate abundance figure.

6.The title should be also changed, because the authors did not show any alleviation of carcinogenesis using VSL#3. They showed that VSL#3.could act preventivelly.

Answer: I have changed the title to “VSL#3 Can Prevent Ulcerative Colitis Carcinogenesis in Mice”.

INITIAL REVIEW OF THE MANUSCRIPT

Google Search:

[ ] The same title

[ ] Duplicate publication

[ ] Plagiarism

[ ] No

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Title: VSL#3 Alleviates Ulcerative Colitis Carcinogenesis in Mice

Reviewer's code: 02941672

Reviewer's country: Japan

Science editor: Xue-Jiao Wang

Date sent for review: 2018-05-28

Date reviewed: 2018-06-10

Review time: 13 Days

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection	Peer-Review: [Y] Anonymous [ ] Onymous Peer-reviewer's expertise on the topic of the manuscript: [ ] Advanced [Y] General [ ] No expertise Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The author had revealed that the preventive effect of 5-ASA and VSL#3 for carcinogenesis by cytokine inhibition in AOM/DSS induced mice model. The VSL#3 alone had anti-tumor effect than combination of 5-ASA and VSL#3. In contrast, 5-ASA alone suppressed cytokine expression than combination of them. The reasons maybe

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unknown, but some hypothesis or explanation need to be described in discussion.

Answer: Thank you for your question. From Table 1, it seems that the VSL#3 alone had anti-tumor effect than combination of 5-ASA and VSL#3, but the *p*-value I showed here is compared with the model group respectively. In fact, if we compared the tumor load of VSL#3 (0.25±0.07 cm) with 5-ASA+VSL#3 (0.46±0.11 cm), there is no statistic significance (p=0.11). Samely, if we compared the IL-6 level of VSL#3 (99.71±31.14 pg/mg tissue) with 5-ASA+VSL#3 (81.43±26.98 pg/mg tissue), there is no statistic significance (p=0.66), either. If we compared the TNF-α level of VSL#3 (25.89±5.25 pg/mg tissue) with 5-ASA+VSL#3 (21.33±4.55 pg/mg tissue), there is also no statistic significance (p=0.52). That means VSL#3 alone has the same effect as combination with 5-ASA both in anti-tumor effect and in suppressing cytokine expression. So I didn't describe it in discussion.

Reviewer's Code: 00049578

Here follows my review of this manuscript.

The paper by Wang et al. reports an in vivo study on the effect of the probiotic mix VSL#3 in the DSS/AOM colorectal cancer model. The authors looked at tumor incidence/size, TNF/IL-6 colonic expression, and the microbiota. They conclude that VSL#3 has a protective effect.

I read this paper with interest. Although the main finding is interesting, the problem is that the experimental design does not allow us to distinguish between antiinflammatory and anticancer effects, and the mechanism remains therefore obscure.

Main comments

1. The model is not standard, normally AOM is associated with several DSS cycles (i.e. the chronic DSS protocol). The fact that it worked makes this less of a problem, but it

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does resemble more the regular (i.e. colitis independent) AOM model. The design is more problematic regarding the examination of mice only after 12 w and giving the treatments all along. This makes it impossible to tell any cancer specific effects from antiinflammatory activity, which is well known. And there is a precedent for VSL#3. Thus the interest of the study is severily limited. Judging from the (rather poor) figure 1, it appears that VSL#3 and 5-ASA did better than the combination during colitis. No significant differences overall, which is rather odd. Then a few fairly big tumors, which is also a bit surprising. Groups are given in the wrong order in Fig. 5. Fig. 4 is of low quality and does not show all groups.

Answer： We conducted a preliminary experiment which I didn't show in this article：compare AOM plus one DSS cycle with AOM plus three DSS cycles, and the results shows that there are no statistic difference in tumor formation, but the mice in one DSS cycle group has low mortality rate，so we choose AOM plus one DSS cycle as our modeling method. We did the examination only after 12w, because we need colon tissue to examine the IL-6、TNF-α and microbiota which need to sacrifice the mice, so multi-point examination is not realistic during the experiment. We know that AOM/DSS model simulate the process of UC carcinogenesis which is thought as the "Inflammation-cancer "transformation, so we speculate VSL#3 prevent UC carcinogenesis by decreasing the inflammatory factors levels. Although there are some research in VSL#3 and UC carcinogenesis, but few focus on the variation of gut microbiota, so our research have a certain value. In fact, if we compared the tumor load of VSL#3 (0.25±0.07 cm) with 5-ASA+VSL#3 (0.46±0.11 cm), there is no statistic significance (p=0.11). That means VSL#3 alone has the same effect as combination with 5-ASA in anti-tumor effect. These few fairly big tumors are showed in model group which is in stark contrast to other groups. I don't think groups are given in the wrong order in Fig. 5. It's a pity that Fig. 4 is of low quality limited by the poor quality of

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equipment. We didn't show all groups in Figure 4, because we just want to show pathology of normal colon tissue and tumor tissue instead of the severity of the pathology.

2. The choice of parameters measured is a major problem with this study. Two mucosal cytokines at a time point were inflammation is of little importance, and where actually there could be no inflammation at all. Nothing in terms of proliferation, apoptosis, differentiation… The focus is instead on microbiota. Two niches are examined, with no support for this choice. The presentation is not very good I am afraid, and it is hard to tell what the results are. Granted, metagenomics data are difficult to handle, but it could and should be better. Also I was somewhat at odds with the mucosal cleaning procedure: first wash, then shake (in the air?). It does not look as a particularly reproducible or defined process.

Answer: We choose IL-6 and TNF-α as the two mucaosal cytokines because they have plenty evidence associated with carcinogenesis which had been confirmed by previous researches. We didn't focus on the proliferation, apoptosis, differentiation，because microbiota is our innovation. As for the mucosal cleaning procedure, we first wash the mucosal to remove feces from the mucous which may interfer analysis of mucosa microbiota, and shake in the air just to remove the water. This does not affect the experimental results. But as you suggested, I have delete the expression "shake" in order to avoid misunderstanding.

3. P2: there is very little inflammation in the absence of microbiota, this was demonstrated by Rakoff-Nahoum et al. as a matter of fact. Thus the comment about carcinogenesis in this context is out of place.

Answer: I agree that there is very little inflammation in the absence of microbiota，but I

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don't think it conflicts with my experimental results. We speculate the probiotics prevent carcinogenesis by decreasing the inflammation level.

4. English needs revision. For instance P1 'lavaged with 5-ASA', 'wight' (Fig. 1), 'showed' 'procession' (P2), 'caliper' (P5), and so forth. English is not disastrous but it does require substantial refining, not necessarily professional. Also it is confusing to use 'treatment group' for 5-ASA and 'model group' for the untreated animals; by any standard VSL#3 is also a treatment.

Answer: Thank you for your advice. I have revised my expressions. And the treatment group in this paper represents the 5-ASA、VSL#3 and 5-ASA+VSL#3 groups, which have treatment on mice.

5. Treatments were provided in drinking water? This means there was no gavage (or 'lavage'). There are no water intake data, which are mandatory in this case. It is not clear when treatments started, it seems they were introduced from day 1. Figure 1 is strangely not proportional. It would be much better to zoom up on the frist 2 weeks, then shrink the remainder, for better clarity. It has to depict the treatments.

Answer: The 5-ASA and VSL#3 are dissolved in drinking water, and we give them to mice by gavage. The dose of the 5-ASA and VSL#3 by gavage is accurate, so we don't need to record water intake data. The treatment were introduced from the day of injection AOM. In Figure 1，the abscissa represents the number of weeks，I'm afraid I should bisecting the abscissa. But I have revised Figure 1 for better clarity.

6. The authors themselves state that VSL#3 treatment did not result in increased presence in the microbiota, this is remarkable although there are precedents. It is perhaps consistent with a diluted effect after early modulation of the microbiota (during

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colitis).

Answer： Our research found that after the intervention with VSL#3, Bacillus and Lactococcus were increased in fecal microbiota. After supplemented with VSL#3, Bifidobacterium were increased in mucosal microbiota.

7. 5-ASA is absorbed in the small intestine, although sufficiently large doses may reach the colon. This has not been touched in the discussion.

Answer： The therapeutic effect of mesalazine is mainly dependent on the contact of the drug with the local intestinal mucosa. We choose PENTASA which can release from duodenum to rectum. So it doesn't affect our result that it is absorbed in the small intestine.

Minor comments

1. No page numbers, therefore no way to tell the authors were to look. I use numbers based on my printout.

Answer: I have added the page number.

2. Separate numbers and units.

Answer： I have already corrected it.

3. It is a stretch to consider DSS an ulcerative colitis model, better to refer to it as inflammation or IBD model.

Answer: There are already have some previous studied published which use DSS as ulcerative colitis model, so I think it's a relatively reasonable model.

4. Avoid the term 'blank' for the control group without disease.

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Answer: I have already corrected it.

5. Fig. 3 has legend in separate page. Under a macroscopic? Light is not optimal here.

Answer: I have already corrected it. Figure 3 is under a macroscopic. I have adjusted the exposure of my photos.

6. Fig. 1: hard to see anything here.

Answer: I have already corrected it.

7. P4: by around 10 weeks?

Answer： 10 weeks+2days accurately, I have already corrected it.

8. Table 2 and 3 are duplicate data, they should be eliminated.

Answer： Table 2 shows colon tissue TNF-α in each group, while Table 3 shows Colon tissue IL-6 in each group, they are not duplicate data.

9. Bacterial names are in the wrong format.

Answer: I have already corrected it.

INITIAL REVIEW OF THE MANUSCRIPT

Google Search:

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[ ] Duplicate publication

[ ] Plagiarism

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