June 27, 2021

Lian-Sheng Ma

Science Editor, Company Editor-in-Chief

Baishideng Publishing Group

Dear Professor Lian-Sheng Ma

Thank you for reporting that the Basic Study Manuscript No. 67530 entitled "Survival effect of probiotics in a rat model of colorectal cancer treated with capecitabine" has been considered for its publication in the World Journal of Gastrointestinal Oncology, after an appropriate review.

We have modified the manuscript in response to the suggestions of both Reviewers and the Science Editor. We appreciate this criticism that has contributed to improve the presentation of our work.

Detailed in the enclosed sheet there is an enumeration of the changes made in our manuscript. We hope that it will be possible for you to find our paper fully acceptable for publication in the World Journal of Gastrointestinal Oncology.

Yours sincerely,

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Comment of Reviewer #1: 1.This is a very good basic research paper with fluent language. 2. From page 10, we know that,"animals received 1,2-DMH at dose of 20 mg/kg body weight intramuscularly weekly for 8 weeks to achieve colorectal cancer in 100% of animals at 20 weeks(140days) from begun the induction."Why did the DMH + Capecitabine group receive Capecitabine at 146 days from begun the induction(see page13, Figure 1)? Does the 6-day delay affect the treatment outcome? 3. Can you further explore the relationship between intervention factors and colorectal tumor location？

Response of Authors:

1. We appreciate the reviewer's opinion regarding that the manuscript is a very good basic research paper with fluent language.

2. To induce carcinogenesis, the animals received 1,2-DMH at dose of 20 mg/kg body weight intramuscularly weekly for 8 weeks. In DMH-C group the capecitabine treatment was started 90 days after the last day of 1,2-DMH administration. This time (90 days) was taken into account to achieve colorectal cancer in 100% of the animals. In view of the query of the reviewer, we consider that the time "20 weeks" (included in the original manuscript, see Materials and Methods Section, carcinogenesis induction (page 7, last sentence) does not precisely define the number of days that elapsed from the first 1,2-DMH injection until the start of capecitabine administration and the reader could have the same concern as the reviewer.

So, to avoid confusion, and to clarify the concern of the reviewer, in Materials and Methods Section, carcinogenesis induction (page 7, last sentence) we delete the time 20 weeks. Based on this change, we now add the following sentence:

Then, and as it was previously established by our laboratory (Gigola et al., 2011), animals received 1,2-DMH at dose of 20 mg/kg body weight intramuscularly weekly for 8 weeks to achieve colorectal cancer in 100% of animals at 90 days after the last 1,2-DMH administration.

3. In our work we found that 70% of DMH group developed cancer in the left colon (distal colon) and 10% in the right colon (proximal colon), in addition to 20% in the rectum. Similar results were showed by Ma and colleagues regarding the tumor location in rats with 1-2 DMH administration. They observed that when total colon was exposed to this pro-carcinogen, 73 % of tumors occurred distally and only 12 % occurred proximally (Ma et al., 1999); furthermore, they suggested that the observed differences between proliferation patterns in distal and proximal colon may be associated with the higher incidence of tumors in the distal colon (Ma et al., 2002)

The available literature describes that tumors in the proximal colon and distal colon show different molecular and histological characteristics; also the therapy responses are totally different between these tumor entities (Baran et al., 2018). CRC patients with tumors in left colon are more benefited with adjuvant chemotherapies such as 5-fluorouracil (5-FU)-based regimes and have a better prognosis while CRC patients with tumor in right colon do not respond well to conventional chemotherapies. According with these data, although we observed in DMH group that the percentage of left colon tumors was 70 %, this percentage is much lower in both DMH groups subjected to capecitabine treatment, DMH-C and DMH-C-P groups (70% DMH group vs. 33% DMH-C group and 22 % DMH-C-P, see Table 1). With respect to DMH-P group, the reduction of the percentage was less remarkable (63%) since the action of the probiotics delays the appearance of tumors, but does not prevent it. In line with this and with the data from Baran and colleagues, the tumor location in the right colon does not vary substantially in all groups. We observed that DMH and DMH-P groups developed rectum cancer. Consistent with the well established benefit of the capecitabine as chemotherapeutic agent in the treatment of rectal cancer patients (Yang et al., 2020), we didn't find tumors in the groups that receiving this drug (DMH-C and DMH-C-P). The relationship between colorectal tumor location and intervention factors such us 1,2-DMH and capecitabine are now discussed in an appropriate comment added in Discussion section (page 14-15, fifth, sixth and seventh paragraph)

It should be noted that the development of rectum cancer in DMH-P group (showed in Table 1) had not been commented in the Results section. For this reason we have now added this data in the Results section, Clinicopathological outcome (page 11, third paragraph)

Comment of Reviewer #2: Why did you take 50 rats for the experiment,and not 100?

Response of Authors:

We appreciate your observation. Before starting the experimental work in rats that is showed in the present manuscript, a preliminary test was carried out using two groups of male rats. Sex was selected according to the literature showing that male rats are more sensitive to 1,2-DMH than female rats (Turusov et al., 1988).

First group: 15 rats received 1,2-DMH at a dose of 15 mg / kg body weight weekly for 6 weeks. In this group 46.6% of the animals developed tumors.

Second group: 15 rats received 1,2-DMH at a dose of 20 mg / kg body weight weekly for 8 weeks. In this group 100% of the animals developed tumors.

In view of this initial assay, it was taken into account the variables of the second group that were used for the statistical analysis and the experimental design of the present work. From this analysis using InfoStat software, it was determined with a significant value (α=0.01) that the appropriate number of rats per group was 10. It should be noted that the number of animals per group in Control and Control plus Probiotics groups was 5 instead of 10 because our previous results revealed that all animals from these groups showed the same behavior without any abnormality or complications and all of them died from natural causes without objective injuries (Gigola G. 2014). So, and in accordance with the National Institutes of Health guide for the care and use of Laboratory animals, we decided to reduce the number of the animals in 5.

Therefore, the number of rats for each group was as follow:

Animal Care House Control group (Control, n=5)

Animal Care House Control group + probiotics (Control-P, n=5)

DMH group (DMH, n=10)

DMH + Probiotics (DMH-P, n=10)

DMH + Capecitabine group (DMH-C, n=10)

DMH + Capecitabine + Probiotics group (DMH-C-P, n=10)

An appropriate comment is now added in Material and Method section, Animal model (page 7, second sentence) and Experimental design and animal trial (page 8, first paragraph).

Comments from the Science editor:

1 Scientific quality: The manuscript describes a Basic Study of the Probiotics effects on colorectal cancer model. The topic is within the scope of the WJG.

(1) Classification: Grade B and Grade B;

(2) Summary of the Peer-Review Report: This is a very good basic research paper with fluent language. Authors should explain if the 6-day delay affect the treatment outcome and further explore the relationship between intervention factors and colorectal tumor location. The questions raised by the reviewers should be answered

Response of Authors:

We have carefully answered the comments of both reviewers and have modified the manuscript in response to their suggestions. The responses to the reviewers were previously listed in this letter. In each response we mentioned the pages and paragraphs where we added the modifications in the manuscript in order to facilitate their visualization.

Comments from the Science editor:

(3) Format: There are 3 tables and 5 figure;

(4) References: A total of 40 references are cited, including 10 references published in the last 3 years;

(5) Self-cited references: There are 2 self-cited references.

Response of Authors:

(3) The revised manuscript still contains 3 tables and 5 figures.

(4) Five references from other authors were cited in response to both reviewers and were added in the revised manuscript. Due to the modifications made in the Discussion section (page 14-15, fifth, sixth and seventh paragraph), and in the Materials and Methods Section, Animal model (page 7, second sentence) we have added the following references:

1- **Baran B**, Mert Ozupek N, Yerli Tetik N, Acar E, Bekcioglu O, Baskin Y. Difference Between Left-Sided and Right-Sided Colorectal Cancer: A Focused Review of Literature. *Gastroenterology Res*. 2018; 11(4):264-273. [PMID: 30116425 PMCID: PMC6089587 DOI: 10.14740/gr1062w]

2- **Ma Q**, Williamson KE, O'rourke D, Rowlands BJ. The effects of l-arginine on crypt cell hyperproliferation in colorectal cancer. *J Surg Res* 1999; 81: 181-188 [PMID: 9927538 DOI: 10.1006/jsre.1998.5512]

3- **Ma QY**, Williamson KE, Rowlands BJ. Variability of cell proliferation in the proximal and distal colon of normal rats and rats with dimethylhydrazine induced carcinogenesis. *World J Gastroenterol* 2002; 8(5):847-52. [PMID: 12378628 PMCID: PMC4656573 DOI: 10.3748/wjg.v8.i5.847]

4- **Turusov VS**, Lanko NS, Parfenov YD, Gordon WP, Nelson SD, Hillery PS, Keefer LK. Carcinogenicity of deuterium-labeled 1,2-dimethylhydrazine in mice. *Cancer Res* 1988; 48: 2162-2167. [PMID: 3349486]

5- **Yang XH**, Li KG, Wei JB, Wu CH, Liang SX, Mo XW, Chen JS, Tang WZ, Qu S. Retrospective study of preoperative chemoradiotherapy with capecitabine versus capecitabine plus oxaliplatin for locally advanced rectal cancer. *Sci Rep*. 2020; 10(1):12539. [PMID: 32719436 PMCID: PMC7385078 DOI: 10.1038/s41598-020-69573-z]

Therefore, in the revised manuscript there are now a total of forty five references.

(5) We keep the self-cited references: Thus, there are 2 self-cited references in the revised manuscript.

Comments from the Science editor:

2 Language evaluation: Classification: Grade A and Grade A. A language editing certificate issued by academia editing was provided.

3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the Institutional Review Board Approval Form. No academic misconduct was found in the Bing search.

4 Supplementary comments: This is an invited manuscript. The study was supported byUniversidad Nacional del Sur. The topic has not previously been published in the WJG. (13)

Response of Authors:

2 3 4 We are pleased that the writing and research topic is in accordance with the expected quality.

Comments from the Science Editor

5 Issues raised: (1) The "Author Contributions" section is missing. Please provide the author contributions;

Response of Authors:

We have now incorporated in the revised manuscript the "Author Contributions" section in the first page of the revised manuscript.

Comments from the Science Editor:

(2) The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s)

Response of Authors:

The corresponding documents have been uploaded to the F6 Publishing system along with the rest of the revision files.

Comments from the Science Editor:

(3) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

Response of Authors:

All figures are original for this work and have not been submitted for another publication. An appropriate text was incorporated into the legend of each figure to clarify this point.

In addition, we prepared and arranged the figures using PowerPoint.

Comments from the Science editor:

(4) The "Article Highlights" section is missing. Please add the "Article Highlights" section at the end of the main text.

Response of Authors:

The highlights section of the article was prepared considering the seven suggested subsections in order to facilitate the approach to the article. The following seven highlights were added to the "Article Highlights" section to the end of the main text in the revised manuscript (page 17).

1-Colorectal cancer (CRC) is one of the leading causes of mortality due to malignant diseases worldwide (Research background).

2-Capecitabine, the prodrug of 5-fluorouracil (5-FU), is one of the most important chemotherapeutic agents used in CRC treatment (Research background).

3-Prolonged use of regimens containing Capecitabine can lead to systemic toxicity with the consequent discontinuation of the treatment (Research background).

4-To improve the management of CRC patients, it is necessary the incorporation of therapies that mitigate the side effects of the conventional CRC treatment and reduce its resistance (Research motivation).

5-Probiotics have beneficial properties when they are used in the management of many gastrointestinal diseases. Also, it is known that probiotics are able to reduce undesirable effects of 5-FU in CRC patients and to benefit CRC patients treated surgically (Research motivation)

6-In a rat CRC model, probiotic supplementation potentiated the antitumor effect of 5-FU chemotherapy on colon (Research motivation)

7-The positive impact of probiotics in a preclinical model of CRC under capecitabine treatment was unknown when we started our experimental work (Research motivation)

8-The aim of this study was to evaluate the impact of a mixture of probiotics strains in the outcome of a rat CRC model treated with capecitabine and monitored until the end of life (Research objectives).

9-Male Wistar-Lewis rats with CRC induced by 1,2-dimethylhydrazine dihydrochloride (1,2-DMH) were grouped as follow: 1.2-DMH alone (DMH group, n=10), 1,2-DMH + capecitabine (DMH-C group, n=10), 1,2-DMH + probiotics (DMH-P group, n=10), 1,2-DMH + capecitabine + probiotics (DMH-C-P group, n=10) (Research methods).

10- Two groups of male Wistar-Lewis rats were used as controls: untreated group (Control n=5) and Control + probiotics group (Control-P, n=5) (Research methods).

11- During the experiment, the following were analyzed in all groups: survival time, clinicopathological characteristics, quality of life and cause of death (Research methods).

12- The administration of probiotics showed a benefit in survival time, weight gain, clinical manifestations and cancer development (Research results)

13- The fact that the animals were followed until the end of life allow to conclude that this study is the first that shows the positive impact of probiotics in the overall survival of rats with CRC under capecitabine treatment (Research conclusions)

14- The use of probiotics could improve the overall survival and quality of life of patients with CRC treated with capecitabine (Research perspectives)

References mentioned in this letter

Baran B, Mert Ozupek N, Yerli Tetik N, Acar E, Bekcioglu O, Baskin Y. Difference Between Left-Sided and Right-Sided Colorectal Cancer: A Focused Review of Literature. *Gastroenterology Res*. 2018; 11(4):264-273. [PMID: 30116425 PMCID: PMC6089587 DOI: 10.14740/gr1062w]

Gigola G, Melatini G, Ullua N, Cardozo C, Martín AG, et al. Comparación de dos protocolos de inducción de cáncer colorrectal en ratas Wistar Lewis con 1,2-Dimetilhidrazina. *Oncología Clínica* 2011; 16(1):1-4.

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Yang XH, Li KG, Wei JB, Wu CH, Liang SX, Mo XW, Chen JS, Tang WZ, Qu S. Retrospective study of preoperative chemoradiotherapy with capecitabine versus capecitabine plus oxaliplatin for locally advanced rectal cancer. *Sci Rep*. 2020; 10(1):12539. [PMID: 32719436 PMCID: PMC7385078 DOI: 10.1038/s41598-020-69573-z]