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Letter to the Review Editor

Date: May, 18, 2016

To: Jing Yu
Science Editor
World Journal of Gastroenterology

From: Ki Tae Suk, M.D., Ph.D. ktsuk@hallym.ac.kr

Subject: Revision for ESPS manuscript NO: 25236

" Microbiota-based Treatments in Alcoholic Liver Disease"

Dear Editor:

First, we appreciate the Editors' and reviewers' thoughtful and helpful comments. Also, we are pleased to have an opportunity to resubmit and make this paper to be an even better one, because the Editor and reviewers provided additional important points that we haven't realized before.

Here, we are resubmitting the revised manuscript that addresses the several concerns of the reviewers. We have included the changes as recommended by the reviewers in the revised manuscript and provided a detailed point-by-point response to all of the reviewers' comments.

We hope that the paper will now be considered for publication because we believe that our paper has important and substantial implications for clinicians.

We thank you for your time and look forward to your reply.

Sincerely yours,

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ESPS manuscript NO: 25236

" Microbiota-based Treatments in Alcoholic Liver Disease "

Point-to-point responses to comments by the **Reviewer 1**

First of all, we would like to thank the Reviewer1 for his/her favorable comments and criticism, which significantly helped us to improve the quality of this manuscript.

Specific Comments:

• Comment 1 & 2: Describe the average amount of alcohol intake and time in the cited studies, which changes were related to enteric flora, if it is the same as described for increased risk of developing chronic liver disease either the quantity and time are larger or smaller. Add a table of the suggested therapies and studies cited for each, with potential benefits demonstrated, so it is more illustrative to readers

.**• Response 1:** We appreciate the Reviewer's thoughtful comment. We made Table 1-3 for the easy understanding to reader. In the table, we describe the alcohol amount or possible enroll criteria for clinical study and alcohol amount and duration for animal study. In case of liver cirrhosis, we described number and percentage of alcohol related cirrhosis.

Table 1. Microbiota-based treatment with probiotics in alcoholic liver disease - clinical trial

Patients	Enroll criteria or alcohol amount	Treatment	Results	Ref
Compensated LC (alcohol: 22 [56.4%]) Age=53 M/F=1.8:1	Liver biopsy Biochemical study Endotoxin level Stool microbiota	*Escherichia coli* Nissle (2.5-25x10^9 for 42 d)	*Lactobacillus* & *Bifidobacterium* sp. ↑ *Proteus hausei & Citrobacter* sp. ↓ *Morganella* sp. & endotoxemia ↓ improvement of liver functions	70
Alcohol-related psychosis (66 [73.3%]) Age=42.3±1.1 All males	Consumed 750mL of Russian vodka (40% ethanol, daily)	*Bifidobacterium bifidium* (0.9x10^8 CFU for 5 d) *Lactobacillus plantarum* 8PA3 (0.9x10^9 CFU for 5 d)	Bifidobacteria & Lactobacilli ↑ AST & ALT ↓	71
LC (alcohol: 12 [48%]) Age=51.2±1.8 M/F=2:1	LC	*Lactobacillus casei* Shirota (19.5x10^9 CFU for 28 d)	Neutrophil phagocytic capacity ↑ sTNFR1 ↓ sTNFR2 ↓ IL10 ↓ TLR4 ↓	72
AH (60 [51.3%]) Age=52.7±11.3 M/F=5.3:1	AST/ALT>1 AST& ALT level ↑ Alcohol intake >40 g/day for female >60 g/day for male	*Lactobacillus subtilis, Streptococcus faecium* (1,500 mg/d for 7 d)	Serum LPS level ↓ TNF-α ↓	74

LC, liver cirrhosis; AH, alcoholic hepatitis; ALT, alanine transaminase; AST, aspirate transaminase; IL, interleukin; sTNFR, soluble tumor necrosis factor; TNF, tumor necrosis factor; M, male; F, female; d, day; LPS, lipopolysaccharide; CFU, colony forming unit

Table 2. Microbiota-based treatment in alcoholic liver disease- animal studies

Animal model	Alcohol amount	Treatment	Results	Ref
6-week-old male 10 C57BL/6 mice	Lieber-DeCarli liquid diet with 10% alcohol for 6 wks	*Lactobacillus rhamnosus* R0011, *Lactobacillus acidophilus* R0052 (1/mg/mL/d for 4 wks)	TLR-4 ↓ IL-1β ↓	75
4-week-old male 20 C57BL/6 mice (10 Normal diet, 10 High-fat diet)	Oral administration 5 g/kg/day, twice/week, for 9 wks	*Lactobacillus rhamnosus* R0011, *Lactobacillus acidophilus* R0052 (1 mg/mL/d for 2 wks)	In normal diet groups TNF-α ↓ IL-1↓ TLR4 ↓ TLR4/GADPH ↓ In high-fat diet groups: IL-10 ↑	76
Male Sprague-Dawley rats	Dose gradually increased every 2 to 3 days up to a maximum of 8 g/kg/day by 2 wks 6 g/kg/d for final 10 wks	Oats (10 g/kg/d)	Tight junctions in colon ↓ Disorganization of actin cytoskeleton ↓ Oxidative stress ↓ NO overproduction ↓ Oxidative tissue damage ↓ Nitrotyrosine ↓ Carbonyl ↓	79

GAPDH, glyceraldehyde-3-phospate dehydrogenase; IL, interleukin; NO, nitrooxidative, TLR, toll-like receptor; TNF, tumor necrosis factor

Table 3. Microbiota-based treatment with prebiotics and antibiotics in alcoholic liver disease-clinical trial

Patients	Enroll criteria or alcohol amount	Treatment	Results	Ref
HE (alcohol 140 [46.8%]) Age=56±10 M/F=1.2:1	≥ two episodes of overt HE (Conn score ≥2) LC (MELD ≤ 25)	Rifaximin (1,100 mg/d, for 6 mo)	Episode of encephalopathy ↓ (hazard ratio: 0.42)	16
LC with subclinical HE (alcohol 36 [48%]) Age=62.0±7.3 M/F=1.2:1	Psychometric tests -Trail making test A -Wechsler adult intelligence scale -Symbol digit -Block design tests	Lactulose (45 mL/d for 8 wks)	Number of the abnormal psychometric test ↓ Prevalence of subclinical HE ↓	80
LC (alcohol 12 [35.3%]) Age=57.6 M/F=0.8:1	Laboratory investigations -Liver biopsy -Endoscopy.	Norfloxacin (800 mg/d) Neomycin (1,500 mg/d) alternating periods of 15 days for 6 mo	Small-intestinal motor activity ↑ Transit time ↓ Small intestinal bacterial overgrowth ↓ Child-Pugh Score ↓	82
TC (alcohol 13 [56.5%]) Age=58±3 M/F=11.5:1	For LC -Liver biopsy -Laboratory findings For hematological indices -Platelet count ≤150,000/μl	Rifaximin (1,200 mg/d, for 4 wks)	Platelet count ↑ Endotoxin ↓ IL-1 ↓ IL-6 ↓ TNF-α ↓	83

HE, hepatic encephalopathy; IL, interleukin; LC, liver cirrhosis; PT, patient; TC, thrombocytopenic cirrhosis; TNF, tumor necrosis factor

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" Microbiota-based Treatments in Alcoholic Liver Disease "

Point-to-point responses to comments by the **Reviewer 2**

First of all, we would like to thank the Reviewer1 for his/her favorable comments and criticism, which significantly helped us to improve the quality of this manuscript.

Specific Comments:

• Comment 1 & 2: (1) The Authors shall distinguish the different stages of ALD and in this context they should present the role of microbiota-based treatments. (2) The Authors shall clarify whether the amount of alcohol used has an impact on the effectiveness of treatment with these substances.

• Response 1: Thank you for your important suggestion. We made Table 1-3 for the easy understanding to reader. In the table, we describe the alcohol amount or possible enroll criteria for clinical study and alcohol amount and duration for animal study. In case of liver cirrhosis, we described number and percentage of alcohol related cirrhosis.

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