AUTHORS' RESPONSES TO THE REVIEWERS' COMMENTS

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Contact litholysis by tert-amyl ethyl ether (TAEE) is superior to MTBE in both *in vitro* and *in vivo* models of gallstones

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We thank the reviewers and the associate editor very much for their insightful and valuable comments. We believe that *World journal of gastroenterology* is appropriate for our manuscript that we intend to be a practical paper that is helpful for future clinical applications. In this document, we quote the reviewers' comments in **bold type**; our replies follow in regular lettering. Moreover, we corrected a few minor improper expressions and grammatical errors that are not specifically mentioned here; we hope that this is acceptable.

Reviewer Name: Anonymous

Review Date: 2019-07-23 11:21

1.How was sample size calculated?

RESPONSE)

Thank you for pointing it to us. Sample sizes in this experiment are as follows.

1) In vitro gallstone dissolubility test

a. Cholesterol gallstone (N = 40): MTBE (n = 20) and TAEE (n = 20)

b. Mixed gallstone (N = 40): MTBE (n = 20) and TAEE (n = 20)

c. Pigmented gallstone (N = 40): MTBE (n = 20) and TAEE (n = 20)

2) In vivo gallstone dissolubility test (N = 44): Hamster model

a. Control hamsters (n = 10)

b. Hamsters with cholesterol stone (n = 15): DMSO (n = 4), MTBE (n = 4), and TAEE (n = 7)

c. Hamsters with pigmented stone (n = 14): DMSO (n = 4), MTBE (n = 4), and TAEE (n = 6)

3) Mouse model of acute toxicity (N = 35): Ct (n = 7), MTBE (n = 14), and TAEE (n = 14)

We have specified this information in the text.

2.How were the animals allocated into groups? (group):

RESPONSE)

We presented it in answer to question 1. The difference in the number of hamsters in each group was due to the difference in the actual number of hamsters that finally had been induced to have cholesterol (n =15) and pigmented (n = 14) gallstones from the diet-starters (N = 17, respectively)

3.Was blinding / randomisation done in the course of the study?

RESPONSE)

We really appreciate your kind remark. We randomized the hamsters for in vivo dissolubility and the mice for the acute toxicity test. We also adhered to the principle of double blindness for in vivo dissolubility testing. The examiners injecting each compound into hamsters did not recognize the solvent he was injecting, and the other examiners measuring *in vivo* dissolubility could not aware of the causable solvent. We mention this in the revised manuscript.

4.How was the volume (0.1ml) of fluid injected for toxicity arrived at?

RESPONSE)

After laparotomy under general anesthesia, the gallbladder was identified, and the bile in the gallbladder was completely aspirated using a 30-gauge syringe. Subsequently, the gallbladder was cautiously filled with a volume (0.1 ml) of dimethyl sulfoxide (DMSO), MTBE, and TAEE, respectively. After 24 h, gallstone dissolubility of each solvent was determined by comparing the weights of solvent-treated gallstones and control (DMSO)-treated gallstones.

For determining the direct tissue toxicity of MTBE and TAEE, we performed cleaved caspase-3 immunohistochemistry using the gallbladder specimens obtained from the hamsters treated with each solvent, respectively. In addition, for determining the toxic effects of MTBE and TAEE on the liver and kidney, we compared the histological findings of the liver and kidney specimens that had been attained from the hamsters at 24 h after infusion of each solvent into the gallbladder. Finally, for determining the effects of MTBE and TAEE on the systemic inflammation, we compared the serum levels of pro-inflammatory cytokines (IL-6 and TNF-α) by ELISA at 24 h after infusion.

5.What were the limitations of the study?

RESPONSE)

This experiment corresponds to *in vitro* experiment and preclinical small animal study, and clinical study is required for future clinical application. The principal limitation of this study

is relatively small number of samples used in this experiment. Regarding *in vitro* dissolubility of each solvent (MTBE or TAEE) for cholesterol gallstones, we determined it by reacting the solvent, respectively, with cholesterol gallstones from obtained from 20 patients, respectively. In the same way, we determined dissolubility of each solvent for mixed gallstones and pigmented gallstones. In determining *in vitro* dissolubility, we treated MTBE and TAEE, respectively, by dividing 40 cholesterol gallstones, 40 mixed gallstones, and 40 pigmented gallstones into two groups. In the *in vitro* dissolubility test, the number of hamsters with cholesterol gallstones and pigmented gallstones were 15 and 14, respectively. However, all the experiments were elaborately performed, and significant results were obtained in spite of a relatively small number of samples. We believe that this study satisfied the fulfillments of preclinical study, and further clinical studies should be directed to overcome the limitations of this study.

Reviewer Name: Anonymous

Review Date: 2019-07-28 16:00

Specific Comments To Authors: To Authors The topic of the manuscript is intersting and has an application and development perspective in the treatment of biliary lithiasis. The idea to dissolve the biliary lithiasis is stimulating even if not new. The study design for the evaluation of a new solvent TAEE and its comparison with the already used MTBE is well developed (Methods) and the Results showed are believable.

It seems to me an error in the Abstract-Results: “in vitro” repeated twice (for both experiences). In the Discussion the topic of gallstones, cholecystectomy and digestive cancer is very enphasized in relation to the therapeutic choice of the biliary lithiasis

dissolution. The therapeutic use of contact litholysis by TAEE is not of next current application. The connection between gallstones, cholecystectomy and digestive cancer is very complex and needs more extended presentation and explication. Based on these considerations, I suggest to reduce this section in the Discussion.

RESPONSE)

We deeply appreciate your compliment and valuable comments. The mistaken expression in the abstract has been corrected as you pointed out (the second “in vitro” in the result section of abstract → “in vivo”). We apologize for the inaccuracies in expressing our results. In addition, you recommended that we reduce the details of the association between cholecystectomy and digestive cancer in the discussion. As you mentioned the relationship between cholecystectomy and digestive cancer is very complex and requires more research, we are willing to accept your appropriate opinion. In the revised manuscript, we have removed all the descriptions relating cholecystectomy with digestive cancers from the discussion.

Once again, we thank you for your response and hope we have been thorough in answering your comments. Your comments have aided us immensely in improving our manuscript. We hope our revision is satisfactory to your high standards and we readily await your next feed-back.