

Answering reviewers

February 21, 2014

Dear Editor

Please find enclosed the edited manuscript in Word format (file name:6288-review.doc)

Title: Effects of bile acids on COX2 in rat model of duodeno-esophageal anastomosis.

Author:Naoki Hashimoto

Name of Journal: World Journal of Gastroenterology

ESPS Manuscript NO:6288

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

1. Format has been updated.
2. Revision has been made according to the suggestions of the reviewer.
 - A. Reviewer comment (02594127): I think this manuscript is interesting. The article is nice. Well written and well presented. Figure 2 is not very good.→I revised Fig2.(macroscopic appearance of a resected esophagus from EDA and Control rats.
 - B. Reviewer comment (02504712) :This is an excellent experimental study which probably adds to the existing literature.
- ① The comment in the results suggestion that the histological features may depend on the volume of reflux contents etc. The volume of reflux contents was not measured in this study and such a comment in the result is totally inappropriate. →I eliminate the following sentence “Miwa et al ¹⁶suggested SCC developed in places distant from the anastomosis compared to ADC. This means that histological features may depend on the volume of reflux contents; small amounts of reflux causes SCC and a large volume of reflux causes ADC”. Moreover,I eliminate “SCC developed in places distant from the anastomosis compared ADC. This means that histological features may depend on the volume of reflux contents;small amounts of reflux causes SCC and a large volume of reflux causes ADC.(page 6)

- ② With regards to the benefit from COX2 inhibitors, this should be a completely different experiment and should not form part of the conclusion. The last two sentences in the conclusion are unnecessary. → I eliminate following sentence in conclusion , "Our results imply that patients with esophageal cancer, whose tumors express COX2 may benefit from treatment with selective COX2 inhibitor. Such therapy curtail the risk of tumor growth being stimulated by gastroesophageal reflux of bile acids. Our results will hopefully provide important clues to the development of new techniques for the prevention and treatment of esophageal cancer".
- ③ The conclusion are a little too strong and need to be toned down a little bit. → I eliminate the following sentence "We believe that our study provides compelling new evidence that bile acids may induce tumor growth via the COX2 angiogenic pathway.

C. reviewer comment (00003826)

- ① Page3 line1, needs a reference for "... killed as described previously"
→ I revised "All of the rats were killed as described previously^[11] .

Reference 11 Fein M, Peter FH, Chandrasoma P et al. Duodeno-esophageal reflux induces esophageal adenocarcinoma without exogenous carcinogen. *J Gastrointest Surg* 1998;2:260-268 [PMID:9841983 DOI:10.1016/S1091-255X(98)80021-8]

- ② Elucidation of figures should be in the text content, not paragraph title. → I revised according to your suggestion.
- ③ Figure 2 needs to show esophagus from both EDA and control animals. → I revised Fig2.(macroscopic appearance of a resected esophagus from EDA and Control rats.
- ④ PCNA labelling needs to be presented, and what is n=? for $75 \pm 5\%$ etc → I add Fig 4 immunohistochemical findings for PCNA in EDA and Control rats.
n.: EDA=27,Control=10
- ⑤ Both bile acid and PGE2 concentration were from what number of animals, presented as mean \pm SD → n:EDA=27,Control=10 Data are expressed as the mean \pm SD of each group
- ⑥ COX2 mRNA and Westernblot are needed to quantitate its expression changes. → I did not perform COX2 mRNA and Westernblot in this experimental study. Because "We observed markedly enhanced expression of COX2 in dysplastic and cancerous mucosa obtained from rats in which an

esophagoduodenal anastomosis had been created. In contrast, COX2 was undetectable in esophageal and duodenal mucosa from the control rats.”(page 8)

- ⑦ In page 7 paragraph 3, the authors stated “In this study, we demonstrate that bile acids” This reviewer does not think there is enough evidence provided in the manuscript. →I eliminate “In this study, we demonstrate that bile acids induce COX2 expression, increased PGE2 production and hyperproliferation in dysplastic and cancer tissues (Fig4).”

3.References and typesetting were corrected.

Thank you again for publishing our manuscript in the World Journal of Gastroenterology.

Sincerely yours,

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