Reviewer 00069130

I have reviewed the study titled "Anti-fibrotic effects of endothelin-A receptor antagonist, ambrisentan, in NASH model mice", by Okamoto T et al. This is an interesting study. The authors report that 'ambrisentan' attenuates the progression of hepatic fibrosis by inhibiting the activation of HSCs and reducing procollagen-1 and TIMP-1 gene expression. According to them it did not affect inflammation and steatosis. No doubt these results are interesting. However, the data is quite preliminary. The authors didn't include simple H&E staining of liver tissue. F4/80 has high background in the test versus control. They authors have not looked into the changes in portal venous pressure/evidence of portal changes/hypertension. There are minor spelling mistakes. Overall, the study is good enough to be published in World Journal of Hepatology.

Reply
Thank you for your suggestions. We have added HE staining photos and have changed to clearer F4/80 immunostaining photos.
We did not investigate the changes in portal venous pressure in this experiment. This problem is beyond our experiment.

Reviewer 00186131
The manuscript is interesting. However, the number of mice is very limited. Therefore, I suggest to extend the experiment to other mice or alternatively the authors have to explain why hey have used only 13 mice.

Reply
As the reviewer states, mice number was small. We could not prepare sufficient number of mice because it was not easy to reproduce FLS-*ob/ob* mice in this short period. Further experiment using other NASH model mice are needed to confirm the effect of ambrisentan. We have changed to "T The present study has some limitations. First, it involved the small number of mice and the relatively short duration of ambrisentan treatment. The present study included only eight ambrisentan-administered mice and five controls and for only 4 weeks. Therefore, examination of large number of mice and longer administration periods is required to validate these results." as a study limitation in page 19.

Reviewer 00053111

Endothelin (ET) can activate hepatic stellate cells (HSCs). In this manuscript, Okamoto et al. demonstrated that ambrisentan, ET type A receptor antagonist, attenuated hepatic fibrosis via inhibiting the activation of HSC, but did not affect hepatic steatosis in NASH model mice. Although the points and logic leading to the conclusion are clear and experiments are well controlled, the number of mice are too small to lead to the conclusion. Especially the number of control mice is only "5". This reviewer is embarrassed to give the sign of acceptance with this small size.

Reply

As the reviewer states, mice number was small. We could not prepare sufficient number of mice because it was not easy to reproduce FLS-*ob/ob* mice in this short period. Further experiment using other NASH model mice are needed to confirm the effect of ambrisentan. We have changed to "The present study has some limitations. First, it involved the small number of mice and the relatively short duration of ambrisentan treatment. The present study included only eight ambrisentan-administered mice and five controls and for only 4 weeks. Therefore, examination of large number of mice and longer administration periods is required to validate these results." as a study limitation in page 19.

Reviewer 00043561

This manuscript reports the results of an experimental study conducted on a mouse model of NASH. The authors found in their experiment that ambrisentan reduced the degree of fibrosis in their model. I have some comments for the study.

1. Abstract: There is no information on HSC activation in the results, thus concluding with HSC activation is not appropriate.
   Reply
   We have added, "which means activated hepatic stellate cells (HSC)" in abstract.
2. Introduction: Correct citation may be more appropriate for the sentence'' Ambrisentan is a selective ET type A receptor (ETAR) antagonist that is approved for treatment of patients with pulmonary arterial hypertension. ETAR antagonists improve liver fibrosis in cirrhotic rats [8], but its effects on NASH have not been reported.'' Rather than a review.
   Reply

We have added a reference "8. **Galie N,** Olschewski H, Oudiz RJ, Torres F, Frost A, Ghofrani HA, Badesch DB, McGoon MD, McLaughlin VV, Roecker EB, Gerber MJ, Dufton C, Wiens BL, Rubin LJ. Ambrisentan for the treatment of pulmonary arterial hypertension: results of the ambrisentan in pulmonary arterial hypertension, randomized, double-blind, placebo-controlled, multicenter, efficacy (ARIES) study 1 and 2. Circulation. 2008 10; 117:3010–3019. [PMID: 18506008]".

3. Methods: There is no ''no-NASH'' arm, why? Please add this to the limitations. What was the source for ambrisentan should be given. Which cell type was stained for alpha-SMA should be given in detail.
   Reply
   As the reviewer says, "non-NASH" arm is desired. However, DS mouse which is the origin of FLS-ob/ob mice was difficult to obtain. This point should be confirmed using other model mice.

We have added "Second, our experiment did not have non-NASH mice arms because we could not obtain DS mice, original mice of FLS-*ob/ob* mice. Therefore, further study is needed using other NASH model mice." as a study limitation in page 19.
We have added "The areas of neutral lipids were measured to evaluate the hepatic steatosis." and "α-SMA immunostaining was used to detect and count the activated HSCs." in page 10.

We have added "ADooQ BioScience, Irvine, CA" in line 19 of page 7.
4.Results: In Figure-1, photos A,B and H,I are not convincing. The text referring to this figure should also be clear in addressing the correct cell type as HSCs. The authors can check and cite the publication ''World J Gastroenterol2007 June 21; 13(23): 3237-3244''.

Reply
Thank you for your advice. We have changed to new photos with a higher magnified photo of α-SMA positive cell.

I believe the manuscript has been improved satisfactorily and hope it will be accepted for publication in **World Journal of Hepatology**. If there are some troubles, I am prepared for responding to you.

Very sincerely yours,

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