

March 6, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (File name: 8864-Manuscript-revised.doc).

Title: The FXR Agonist GW4064 Alleviates Endotoxin-Induced Hepatic Inflammation by Repressing Macrophage Activation

Author: Jun Yao, Chun-Suo Zhou, Xiong Ma, Bai-Qing Fu, Li-Sheng Tao, Miao Chen, Ya-Ping Xu

Name of Journal: *World Journal of Gastroenterology*

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

Responses to the reviewer:

1. It is not clear how many mice were used for each experimental group.

There were five mice in each group.

2. Mice on a normal diet should also be treated with LPS.

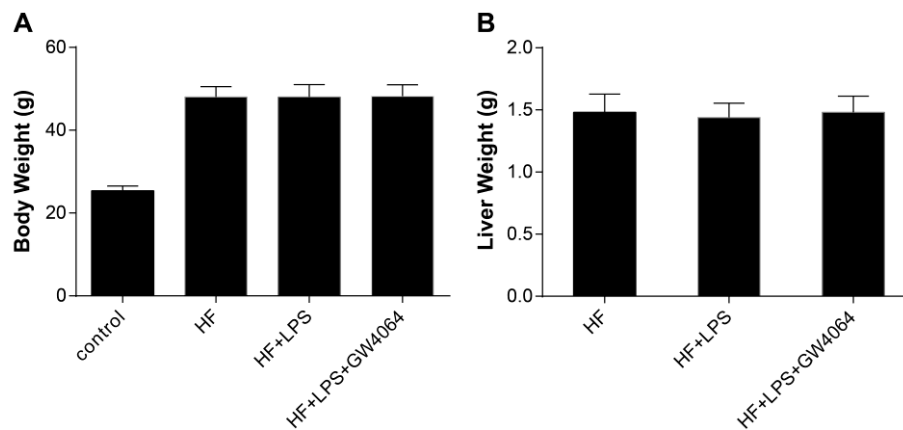
We agree that this is an appropriate control but we did not do this because we have not enough time to do it.

3. It is better to say normal diet instead of control.

We have modified the text accordingly.

4. The authors did not measure body and liver weight of the mice. This is quite important for a study about NAFLD and has to be added.

We did measure the body and liver weight of mice in the experiments, but because there were no significant differences, we did not show the results. We apologize for this omission and have now included the results in the Supplementary Data (Supplementary Figure 1).



Supplementary Figure 1. The body and liver weight of all mice

5. *What about macroscopic changes of the liver? Do you have pictures?*

We did not take pictures of the liver because there were no macroscopic changes.

6. *Why did the authors show no glucose, insulin, or lipid levels of the mice?*

In this study, we focused on inflammation, so we did not measure glucose, insulin, lipid levels. Additionally, the animals in this study were only exposed to GW4064 and LPS for a short period. Furthermore, a about GW4064 in mice fed with a high-fat diet [Pharm Res. 2013 May;30(5):1447-57] showed that synthetic GW4064 prevents diet-induced hepatic steatosis and insulin resistance.

7. *There is no pathologist as a coauthor.*

We apologize for this omission. Chen Miao, from the Department of Pathology of Zhenjiang First People's Hospital, participated in this study.

8. *How was it possible to get "normal" liver tissues? There is no information about the ethics committee regarding this point.*

The “normal” liver tissues were obtained from the tissues adjacent to the hepatic hemangiomas upon resection. The experiments were approved by the Ethics Committee of the First People’s Hospital of Zhenjiang and all patients signed an informed consent form.

9. What is the source of the cell line used?

RAW264.7 cells were purchased from the Shanghai Institute of Cell Biology (Chinese Academy of Sciences).

10. How often were the measurements repeated?

We repeated the experiments three times.

11. No information in Materials & Methods. - Figure 1: The description of the histology is limited. Any changes of the bile ducts, etc.?

We apologize for the limited description. There were no changes of the bile ducts in this experiment.

12. Figure 2: Apoptosis should be additionally analyzed by a second technique (e.g., Western blot)

We have modified the figures accordingly.

13. Figure 3: Change the y-axis to 10 in all figures.

We have modified the figures accordingly.

14. Figure 4: How do the cells look after “treatments”? Any differences? Pictures ?

We did not observe morphologic changes in the RAW264.7 cells following treatment with or without GW4064 nor did we observe differences between the two groups. For this reason, we did not include pictures in the manuscript.

15. Figure 5: Was the cytokine panel measured in human tissues? In addition to the staining for FXR, it would be

necessary to show expression by another method (e.g., Western blot, RNA).

All human tissues were fixed in formalin and embedded in paraffin, precluding cytokine measurement.

16. Table 1: Primer information can be shifted to the Materials & Methods.

We have modified the text accordingly.

17. Table 2 is missing information about BMI, insulin levels, lipids, etc.

We have added this information.

*18. There is also no explanation for * in the table itself.*

This was a clerical error; we have now removed this from the table..

19. There is no citation of Figure 4 in the Results.

We have corrected this mistake.

We have revised the manuscript in line with all the reviewers' comments and we hope that the manuscript is now acceptable for publication at *World Journal of Gastroenterology*. If you have any questions, please feel free to contact us. We appreciate your support very much.

Yours sincerely,

Ya-Ping Xu