

Format for ANSWERING REVIEWERS



JUNE 4, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 10132-review.doc).

Title: GW4064, an FXR agonist, upregulates adipokine expression in preadipocytes and HepG2 cells

Author: Xiaomin Xin; Muxiao Zhong; Gongli Yang; Yao Peng; Yali Zhang; Wei Zhu

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 10132

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

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) In the abstract, the mention conclusion of the work is a repeat of the results and should be omitted.

Responses: The conclusion has been modified accordingly.

(2) The introduction helps in understanding the pathogenesis and symptoms of NAFLD and NASH, however there is a certain lack of knowledge in the role of adiponectin, leptin and resistin in the above mentioned liver conditions. This should be clearly indicated as it will tie up the studies between adipocytes and liver cells. Has there been any studies on the adipokines issued from fatty liver in humans or animal models?

Responses: We thank the reviewer for this comment. Since we have discussed the current state of knowledge regarding adipokines and their receptors in the Discussion section we would prefer not to discuss it in the Introduction.

(3) While the authors aim to find out a therapeutic approach for NAFLD from this study, there is no mention of any other therapies that are currently being used or the lack of it.

Responses: In the article we stated, “Presently, the predominant treatment for NAFLD consists of weight loss with lifestyle modifications because no drugs have yet been approved by International Agencies”. Because there are not yet effective drugs, we would prefer not to indicate the therapies for NAFLD.

(4) There is no justification provided for using different time points for the in-vitro experiments. While 3T3L1 is treated once at day 0, HepG2 cells are treated at an interval of 12 hours for 48 hours.

Responses: Firstly, we wanted to know the effect of FXR agonist on the progress of the differentiation of 3T3-L1 adipocytes, and the change of adipokines and their

receptors after the treatment of GW4064. We used GW4064 at day 0 of the differentiation until day 8 and chose an interval of 48 h. However, in HepG2 cells, we only wanted to know the effect of GW4064 on the adipokines and their receptors at the mRNA levels, and considered that the synthesis and degradation of mRNA just needed a few hours. Since cells no longer in a good state after two days in the six-well plates, we chose the time points of 0, 12, 24 and 48 h.

(5) Any published reference indicating stable expression of GAPDH. Or else other reference genes should be included and used for normalization of the qPCR data.

Responses: The following references are in accordance with the Reviewer's comment:

“Rizzo G, Disante M, Mencarelli A, Renga B, Gioiello A, Pellicciari R, Fiorucci S. The farnesoid X receptor promotes adipocyte differentiation and regulates adipose cell function in vivo. *Mol Pharmacol* 2006; 70: 1164-73.” This article used GAPDH as a reference gene in 3T3-L1 cells.

“Koh I, Lim J H, Joe M K, et al. AdipoR2 is transcriptionally regulated by ER stress

- inducible

277(10): 2304-2317.” This article used GAPDH as a reference gene in HepG2 cells.

”Barber R D, Harmer D W, Coleman R A, et al. GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues[J]. *Physiological genomics*, 2005, 21(3): 389-395.”

In the above article, it is also mentioned that GAPDH was one of the most commonly used housekeeping genes for comparisons of gene expression data, and is stably expressed in the cells. We have mentioned just two of the many published

articles that have used GAPDH as a reference gene.

(6) Figure 3 B shows a significant difference between 0 hrs and 12 hrs which is not clear from the graph. Visually, due the SE bar, they don't look significant different.

Responses: We used a *t*-test to compare between 0 and 12 h; further analysis has confirmed the significant difference.

(7) While there is a justification provided for using 3T3L1 cells in discussion, there is no mention of HepG2 cells. Authors should also justify the selection of HepG2 cell model over others.

Responses: We thank the reviewer for this comment. HepG2 cells are a well-recognized in vitro model of liver cells. The expression of AdipoR2 and OB-Rb being high in HepG2 cells, it appeared appropriate to use this model for describing AdipoR2 and OB-Rb changes after treatment of GW4064. Moreover, several studies have used HepG2 cells: “Koh I, Lim J H, Joe M K, et al. AdipoR2 is transcriptionally regulated by ER stress - inducible ATF3 in H epG 2 hum an hepatocyte cells [J]. FEBS journal, 2010, 277(10): 2304-2317”. As a result, we chose HepG2 cells as an adequate in vitro model of liver cells.

(8)The Minor concerns: 1) There is a scope for the introduction to be shortened that will make it easier for readers to focus on the issues. 2) Use of punctuation marks and space after each word needs to be checked. 3) et al vs et al., should be checked.

Responses: The revised version takes into account these three items.

3 References and typesetting were corrected

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

Sincerely yours,

A handwritten signature in blue ink that reads "Wei Zhu". The letters are cursive and fluid.

Wei Zhu, PHD

Guangdong Provincial Key Laboratory of Gastroenterology, Department of Gastroenterology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, China

Fax: +86-20-87280770

E-mail: chnz_w@126.com

A handwritten signature in black ink that reads "Xiaomin Xin". The characters are bold and somewhat stylized.

Xiaomin Xin, M.D

Guangdong Provincial Key Laboratory of Gastroenterology, Department of Gastroenterology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, China; Department of Gastroenterology, Anyang People's Hospital, Anyang 455000, Henan Province, China