ANSWERING REVIEWERS

Janurary 14, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7345-review.doc).

Title: Zinc Protoporphyrin IX Enhances the Chemotherapeutic Response of Hepatoma cells to Cisplatin

Author: Yang-sui Liu, Huan-song Li, Dun-feng Qi, Jun Zhang, Xin-chun Jiang, Kui Shi, Xiao-jun Zhang and Xin-hui Zhang

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7345

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

Reviewed by 02493079

Reasoable conscept Well designed Appropriately written

Reviewed by 01560721

1.The authors used three kind of human liver cancer cell lines; Hep G2, SMMC7721, and 97H. Could you show us clearly which cell line is the resistant cell to CDDP? The authors stated that the HepG2 cell line with the highest HO-1 expression was significantly more chemoresistance to cisplatin than the other cell lines with lower HO-1 expression ($P < 0.05$). Were there significant differences in several doses among three liver cancer cells? And the authors state that the levels of HO-1 protein were detected significantly higher in HEPG2 cell line than in other cell lines in Western blots. However, I think that there was no significant difference in the levels of HO-1 protein between HEPG2 and 97H. You should describe these data clearly.

HO-1 was significant differences expression in three liver cancer cells, HepG2>97H>SMMC7721 ($p<0.05$) .

2. How is the difference of HO-1 expression between the liver cancer cell and normal liver (hepatocyte, hepatic stellate cell or Kupffer cell)?

I am sorry, I did not do this research. But some research suggested that HO-1 was low expression in normal liver.

3. In vivo analysis, the authors showed the analysis of tumor weights. Could you show us the tumor size, tumor numbers and the morphology of tumors? Did you check the apoptosis of tumors by Tunel stain?

Mice were inoculated subcutaneously into the right flanks with 1×107 tumor cells, resuspended in 0.2 ml of PBS. There was one tumor every mouse. Tumor size was difficult to measure. So, tumors were weighed only. I will check the apoptosis of tumors by Tunel stain in future research.

4. In FACS analysis, the authors showed almost same results between CDDP and CDDP+ZNPPIX, CDDP+Hemin. I do not understand the differences between CDDP+ZNPPIX and CDDP+Hemin.

Please show us more clearly results.

Experiments were completely randomized in design and repeated six times. The results suggested ZnPP IX increases cell apoptosis after exposure to cisplatin in comparison with the control group, whereas increased expression of HO-1 by hemin results in decreased cell sensitivity to Cisplatin (4B). CDDP+ZnPP IX>CDDP>CDDP+Hemin($p<.05$)

……

5. In conclusion, the authors state that administration of HO-1 inhibitors may evolve new liver cancer treatment strategies. However, you did not describe the side effects of HO-1 inhibitors. You should describe the merit or demerits of HO-1 inhibitor. Therefore, you should investigate the effect of HO-1 inhibitor in normal liver or cirrhotic model in vitro and in vivo. In addition, is it possible to use ZnPP IX, HO-1 inhibitor as a clinical trial in cirrhotic patients with HCC?

I think the effect of HO-1 inhibitor in normal liver or cirrhotic model in vitro and in vivo is very complicated. It is hard to describe the side effects of HO-1 inhibitors. I will do this research in future.

Minor points 1. On page 5, line 1, the words "in vivo" should been changed to "in vitro".

I am sorry, I had revised as your suggestion.

2. The authors state that the over expression of HO-1 may be associated with chemoresistance to CDDP. In page 5, the authors state that the expression of HO-1 was significantly increased in all liver cancer cells with higher dose of CDDP. However, the high dose of CDDP inhibited all liver cell lines. You should explain the findings of these results.

In discussion, I had described HO-1 is known to be highly induced by a vast array of stress-inducing stimuli such as numerous chemotherapeutics.

3. On page 6, line 4, the authors state that ZnPP IX not only deceased HO-1 activity, but also decreased CDDP induced HO-1 activity ($P<0.05$). The authors show clearly us the difference in HO-1 activity among several groups. 4.

ZnPP IX not only decreased HO-1 activity, but also decreased CDDP induced HO-1 activity, ZnPP IX>CDDP ($p<.05$).

On page 6, line, the sentence "In addition, hemin treatment significantly increased cell apoptosis induced by CDDP" should be changed to "In addition, hemin treatment significantly decreased cell apoptosis induced by CDDP". 5. On page 8, line 14, the sentence "but also increased CDDP (10 μg/ml) induced PROS in liver cancer cell lines" was not understood. Please show us clearly. 6. In Fig 6, the authors showed statistically significant differences. What did you compare? 7. On page 10, line 13, Fig 1 is a mistake.

I am sorry, I had revised as your suggestion.

Reviewed by 02493079

1. All the experiments in this study should be reproducible at least three independently. Their n number and statistical analysis should be clearly described in each figure and legend.

I am sorry, I had revised as your suggestion.

2. The labels in the figures 1B and 4A are too small to identify specific cell lines and treatments.

I am sorry, I had revised as your suggestion.

3. The band density of the Western blot (Figs 1A and 2) should be quantified, normalized, and statistically analyzed.

I had analyzed and compared the western blot by tools, and I think picture is not clear. If it is required, I can provide.

4. Why does the xenograft experiment use female but not male mice? The tumor sizes for each group and treatment at different time points (before drug treatment) should be shown. Although the authors claim HepG2 cells have a higher HO-1 level and thus more resistant to cisplatin treatment than the others, the results of xenograft experiment seem not to show in Figure 7. Why?

I use the same sex for comparison. The tumor sizes is difficult to measure and I will do in future research. I had noticed the results of xenograft experiment, I think that there were some different *in vitro* and *in vivo*.

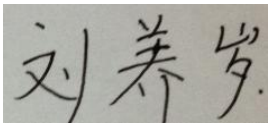
5. The authors claim the resistance to apoptosis by upregulation of HO-1 is related to increased ROS level. The role of ROS in this mechanism should be evaluated in this study

I am sorry, I had revised as your suggestion.

3 References and typesetting were corrected

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

Sincerely yours,



Liu YS, MD
Xuzhou Hospital Affiliated to Medical College of Dongnan University (The Central Hospital of Xuzhou City), Xuzhou, 221009,China
Telephone:+ 86-516-83956131 Fax: 86-516-83956131
E-mail: yangsui-liu@163.com