10th December, 2012

Dear Editor and reviewer:

We greatly appreciate the timely, thorough, and thoughtful reviews of our manuscript (NO: 383). We have revised the manuscript, and would like to re-submit it for your consideration. Please find the edited manuscript enclosed in Word format (file name: 383-review.doc). Point by point responses to the reviewers’ comments are listed below this letter.

**Title:** S. japonicum eggs could concentration-dependently up-regulate the fibrogenesis and apoptosis of primary hepatic stellate cells

**Author:** Ping Liu, Mi Wang, Xiao-Dan Lu, Shu-Juan Zhang, Wang-Xian Tang

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 383

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

1. The English language needs careful proof-reading.

This manuscript had been edited and proofread by American Journal Experts. Please find the editorial certificate in Word format. The title was instead of 〝**S. japonicum egg antigen up-regulates fibrogenesis and inhibits proliferation in primary hepatic stellate cells in a concentration-dependent manner**〞.The running title was 〝**S. japonicum eggs on fibrogenesis and proliferation in hepatic stellate cells**〞. The references were edited according to the BPG’s revision policies for original article in the revised manuscript. The figures were decomposed and added after the manuscript.

2. The use of control animal numbers should be same as the treatment group. Hence, the number of control animals should be increased to 10, instead of 6.

Another 4 normal BALB/C mice were sacrificed and Masson staining pictures of liver tissues were taken. The ratios of collagen fiber area to the total area were evaluated. The new statistical data was showed in the following table and the resubmitted manuscript:

|  |  |  |
| --- | --- | --- |
| **Group** | ***n*** | **The ratio of deposited collagen fiber area against the total (%)** |
| **normal** | 10 | 5.18±1.88 |
| **infected** | 10 | 14.53±2.90a |

3. Once, HSCs were isolated from the control animals, how many days the cells were cultured on plastic plates, before they were incubated with the egg antigen?

Primary HSCs from passages 7-8 (about 3-4 weeks after isolation) were used in this study.

4. a-SMA is expressed only by activated stellate cells. Here, the data has been shown that both control animals and the fibrotic animals express this protein. This contradicts the previous reports.

We appreciate the carefulness of reviewers’ comments. It is true that primary HSCs will be activated when they are cultured in the plastic plate with the increased expression of a-SMA and desmin. In our experiment, since all the primary HSCs were isolated from schistosomiasis-associated liver fibrosis (SSLF) mice, these HSCs had already been activated in vivo. Therefore, the control cells and tested cells were all isolated cells in activated status.

5. The quiescent HSCs store vitamin A deoplets and they tend to flouresce green. This should be detected for the HSC populaiton.

It is true that the quiescent HSCs store vitamin A droplets and tend to fluoresce green, however the content of vitamin A will decrease after activation, and as the result, the green fluorescence will tend to disappear, and on the contrary desmin and a-SMA will increase instead. We used isolated cells which had already been activated in vivo, so it is more rational to use α-SMA and desmin as the markers for activated HSC identification.

6. High doses of the egg antigen causes apoptosis in HSCs. Hence it is possible this dose may kill any kind of cell. Hence, the LD50 should be determined and the data should be presented.

**In vivo**, LD50 (Lethal Dose, 50%) is an index testing the toxicity of poison or drug, which means the concentration of the poison or drug can kill half the test animals. **In vitro**, IC50 was defined as the concentration of stimulants attenuated cell survival to 50%. In our experiment, the schistosomiasis egg antigen is added to the primary HSCs **in vitro**. So in revised manuscript, we determined inhibitory ratios of schistosomiasis egg antigen by cell counting kit-8 (CCK-8) and IC50 was analyzed by SPSS software 16.0 with the inhibitory ratios. In this study, IC50 of schistosomiasis egg antigen was 244.53±35.26μg/ml, and inhibitory ratios of serial concentrations of S.japonicum egg antigen (1, 5, 25, 125, 250, 625, 1250, 2500, 3125μg/ml) was showed in the following figure. In addition, to exclude the cytotoxicity of high concentration egg antigen, we deleted all the contents on the concentration 500 μg/ml in HSCs and the flow cytometry. Please find the relevant contents in the edited manuscript.



7. Authors have elucidated the MAPK/JNK pathway only. If they have to conclude that this pathway is modified not other pathways, they should also check other pathways, e.g. PI3K/Akt pathway etc.

In our study, we try to explain the possible relationship between the MAPK/JNK pathway activation and fibrogenesis induced by S. japonicum egg antigen in activated primary HSCs. Thank you for pointing it out. that PI3K/Akt pathway may play a role. We had added the relevant experiment on PI3K/Akt pathway in the revised manuscript. P-AKT expression was determined by Western-blotting, and the results showed that only when the concentration of S. japonicum egg antigen reached (maybe greater than) 250μg/ml, phospho-AKT (P-AKT) was just activated, which may suggested that AKT signaling pathway may not play as important roles as P38/JNK MAPK in the mechanism involved in the fibogenesis effect induced by S.japonicum egg antigen. The ratios of P-AKT gray scale to that of total-AKT (T-AKT) (P-AKT/T-AKT) was showed in the followed figure:

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

Sincerely yours,

Wangxian Tang

Institute of Liver Diseases, Tongji Hospital

Tongji Medical College, Huazhong University of Science and Technology

1095# Jie-Fang Avenue, Wuhan, China

Phone: +86-27-83662873

FAX: +86-27-83662640

E-mail: tangwx@tjh.tjmu.edu.cn