Author's response to reviews

Title: Cyproheptadine, an Antihistaminic Drug, Inhibits Proliferation of Hepatocellular Carcinoma Cells by Blocking Cell Cycle Progression through the Activation of P38 MAP Kinase

Authors:

Yu-Min Feng (fengyumin2@gmail.com)
Chin-Wen Feng (feng1584@gmail.com)
Syue-Yi Chen (cych06246@gmail.com)
Hsiao-Yen Hsieh (hsiaoyen.hsieh@gmail.com)
Yu-Hsin Chen (b0912070057@gmail.com)
Cheng-Da Hsu (cych06390@gmail.com)

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Yu-Hsin Chen (b0912070057@gmail.com)
Cheng-Da Hsu (cych06390@gmail.com)

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Author’s response to reviews: see over
We would like to thank Prof. Hsu for his insightful review and his interest in our manuscript. We have incorporated all of his recommendations and suggestions in the following manner:

Major compulsory revision
1. The authors argued that cyproheptadine may be preferentially taken by the liver, based on previous case studies. However, excellent treatment response was seen in HCC patients with lung metastases. Therefore, the discrepancy between the concentration tested in vitro and the achievable levels in the clinic remains unsettled.

**RESPONSE:** We thank the reviewer for pointing out this issue. We realize that achievable antitumor concentrations in the human body are an important issue. However, little is known of the pharmacokinetics of cyproheptadine in humans. In regard to serum levels of cyproheptadine, daily treatment with cyproheptadine could produce serum levels of the drug that are higher than those observed after a single dose because plasma cyproheptadine metabolites have a half-life of about 16 hours (Clin Pharmacokinet. 1985, 10:477-497), which may indicate a slow drug elimination process. With respect to cyproheptadine levels in tissues, two independent post mortem case studies (J Anal Toxicol. 1998, 22:72-74; J Anal Toxicol. 2009, 33(8):564-567) found tissue concentrations of cyproheptadine exceeding serum concentrations by up to 3 to 16 times, suggesting large-volume and extensive distribution of cyproheptadine into tissues; among different tissues measured, the highest concentrations of the drug were found in liver and bile, suggesting a possible liver sequestration of the drug. Furthermore, the cyproheptadine concentration in bile could reach as high as 30.7 mg/L (106.8 µM) (J Anal Toxicol. 2009, 33(8):564-567), which is more than twice the concentration of cyproheptadine required to produce an antitumor effect in our *in vitro* study. In view of these results, antitumor concentrations of cyproheptadine in human tissues might be expected to be attained after daily high-dose treatment. This information is now included in the Discussion section (pp. 20–21).

Regarding evidence for the drug’s potential as a treatment option for liver cancer, we conducted a retrospective study to evaluate the efficacy and safety of sorafenib plus cyproheptadine relative to sorafenib alone in patients with advanced HCC. Our unpublished results indicate that cyproheptadine in combination with sorafenib prolongs the overall survival (univariate analysis p = 0.021, multivariate analysis p = 0.002) and progression-free survival (univariate analysis p = 0.017, multivariate
analysis p < 0.001) of sorafenib-treated advanced HCC patients. The percentage of patients with partial response or stable disease in the combination group was twice that in the control group (40% vs. 20%). Strikingly, the probability of sorafenib dose reduction was reduced by approximately half in the combination group compared with the sorafenib control group (22% vs. 40%). The reduced frequency of dose reduction observed in the combination group could be explained by possible alleviation of the adverse effects of sorafenib by cyproheptadine. This pilot study showed that the addition of cyproheptadine to sorafenib results in enhanced improvements to overall survival and progression-free survival in patients with advanced HCC.

2. Thalidomide has both anti-angiogenic and immune modulatory effects. It is not surprising that in vitro tests of HCC cells can not show significant cytotoxic or anti-proliferative effects.

**RESPONSE:** The reviewer is right in pointing out that thalidomide has both anti-angiogenic and immune modulatory effects. However, only animal experiments can address these aspects. In this *in vitro* cellular study, our aims are to investigate the effect of cyproheptadine on HCC cell growth, the cell cycle, and the underlying mechanisms involved in cell cycle arrest.

**Comments from Prof. Mao** (Referee 2)

We would like to thank Prof. Mao for his insightful review and his interest in our manuscript. We have incorporated his recommendation in the following manner:

Minor essential revisions:
Please replace "MicroM" to "µM" other than "uM" as shown in the 1st version.

**RESPONSE:** We appreciate the correction. All measurement units that appeared as "microM" in this manuscript have been changed to “µM”.