Reviewer's report

Title: Up-regulation on Cytochromes P450 in Rat Mediated by Total Alkaloid Extract from Corydalis yanhusuo

Version: 3 Date: 12 June 2014

Reviewer: LIK VOON KIEW

Reviewer's report:

1. Is the question posed by the authors well defined?

   Yes. In the current study, the authors have attempted to assess the potential of total alkaloid extract (TAE) from YHS to effect the activity and mRNA levels of five cytochromes, namely CYP2E1, CYP3A1, CYP1A2, CYP2C11 and CYP2D1 in liver, lung, kidney and intestine, using rat as a model.

2. Are the methods appropriate and well described?

   The description on the method was found to be significantly improved in the latest edition. Nevertheless, there are still a few issues to be addressed, as described in the “suggestion for Major Compulsory / Minor Essential Revision” sections for details.

3. Are the data sound?

   The data was generated from tissues collected at the end of a 2 weeks treatment period. As there is no tissue collection performed along the 2 weeks TAE treatment period, the tissue CYP activities and related mRNA expressions of rats along the treatment period was not known. This has added uncertainty to the results reported, i.e. inability to tell if the elevated / suppressed CYP mRNA expression at the end of the study were due to the consistent TAE treatment, or any event that took place prior to tissue collection; for example, the use of urethane for rat anaesthesia (Meneguz et al., 1999). Comments/justification from the authors on the absence of such data would be important.

   Also, it would be important for the authors to justify the use of urethane in the study, as urethane has been previously reported to cause influence to the liver CYP activities (please see “suggestion for Major Compulsory Revision” section for details.

   Ref:


4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
Please refer to the comments in the “suggestion for Major Compulsory / Minor Essential Revision” sections for further improvements.

5. Are the discussion and conclusions well balanced and adequately supported by the data?

In the discussion, the author has described the potential induction of the CYP2E1 and CYP3A1 enzymes by TAE and indicate the risks of drug-herb interactions following co-administration of Corydalis yanhusuo and drugs. The write-up in the discussion was found to be improved in the current edition of the manuscript.

6. Are limitations of the work clearly stated?

The limitation in the use of rat CYP3A1 in representing human CYP3A4, as well as the possible influence to the liver CYP activities following the use of urethane were not discussed.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?

The authors has attempted to correlate the current findings to that of the published literatures. Nevertheless, most of the references cited for comparison and discussion are of studies involving human subjects or human cell lines, in which differences in activity and range of substrate may occur between CYPs analogues of human and rats (e.g. human CYP3A4 and rat analogue CYP3A1). Parallel comparison with other rat based in vivo studies was not found. It would be useful if the authors can provide some information on this.

8. Do the title and abstract accurately convey what has been found? Yes.

9. Is the writing acceptable?

The grammar of the writing was acceptable.

Major Compulsory Revisions

Method section:

1. Animal experimental procedure:
   a. CMC control group: please provide information on the amount of CMC administered
   b. Rat sacrificial procedure: urethane was used prior to the rat euthanasia. Previously, Menequz et al (1999) has reported elevation of the liver CYP1A and CYP2E1 activities, and the suppression of liver CYP3A activity subsequent to urethane administration to the SD rats (see reference below). Thus, it would be great if the authors can provide some answers / comments / clarifications to the following:
      i. What are the dose of urethane administered in the current study?
ii. Does the urethane treatment contribute to the increase of CYP2E1 and CYP1A2 activities observed in the current study? Please further elaborate / explain your answers.

iii. Does the urethane treatment affect the accuracy of the observed results of CYP3A1 activity in the current study? Please further elaborate / explain your answers.

Ref:

2. Microsomal CYPs activity detection
a. Only rat liver was mentioned for microsome isolation. What about the rest of the tissue collected?

b. How much tissue was used for the preparation of microsomal protein isolates?

3. Minor Essential Revisions

Discussion section:

CYP2E1 induction associated with TAE toxicity

Paragraph 3 line 8: please consider to rephrase the sentence “when these exceed the cellular detoxification systems” to improve clarity.

Induction ability of TAE on CYP3A1 in rats

Paragraph 1 last sentence: Please give further explanation/ clarification on this sentence: “Consumption of YHS or YHS-containing products with CYP3A-inducing compounds should be taken considered because of the possibility of drug–drug interactions.”

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests