Reviewer’s report

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

Version: 2 Date: 5 May 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 is rather vague. For instance, the treatment regimen for various rat groups and the number of animals per treatment group was not clearly defined. Also, the author may want to justify the use of intraperitoneal dose of phenobarbitone as positive control while administrating the test compound via oral route. The (blank/solvent?) control group’s route of administration was not specified as well. The method for the microsomal CYP activity detection was not described clearly, particularly on the LC-MS/MS protocols and the results interpretation. While the author indicated the use of one way ANOVA for statistical analysis, the type post hoc test employed was not mentioned. The above mentioned has made difficult the evaluation of the reliability of the experimental results reporting and interpretation. In addition, the method employed for the culling of rat and blood sampling prior sacrifice (which is important for the animal ethic’s concern) was not mention clearly. The reference number of the animal ethics approval was not provided.

3. Are the data sound?

Although the report on the CYP induction by the TAE was found to be interesting, the evaluation on the reliability of the experimental results reporting and interpretation was not possible without a clear description of the experimental approach. Also, 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 CYP mRNA expression at the end of the study were due to the consistent TAE treatment, or any anonymous transient event that occurred prior to tissue collection).

4. Does the manuscript adhere to the relevant standards for reporting and data
The method reporting are rather crude and need major improvements. Please refer to the comments listed under question 2 and in the “suggestion for Major Compulsory / Minor Essential Revision” sections for details.

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. While rat model has been recommended as a good model for liver microsomal metabolism dependent on CYP2E1 and CYP1A2, Zuber et al. (2002) has suggested that rat may not be a good model of liver microsomal metabolism dependent on CYP3A4. Please refer to the published works of Zuber et al. (2002) for further details. The current results on the elevation of CYP3A1 will be meaningful if the author can provide some examples of the clinically important drugs that are metabolised by both the rat CYP3A1 and human CYP3A4 (with relevant references), or provide references on the CYP3A1 metabolism of drug examples mentioned in the discussion.

Reference:


6. Are limitations of the work clearly stated?

The limitation in the use of rat CYP3A1 in representing human CYP3A4 was not discussed.

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

The authors have 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.

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:
   a. Please provide the reference number for the ethics approval granted by the institutional animal care and use committee (IACUC), i.e. in this case, the Tianjin University of Traditional Chinese Medicine Animal Ethics Committee.

2. Animal experimental procedure:
   a. The information on the number of animal per treatment groups (n number) was not found.
   b. 2 weeks oral administration of TAE: no information on the frequency of the administration / time interval in between administration
   c. The test compounds were orally administered while the positive control was administered i.p. Please provide justification / reference for the use of i.p. injection method for the administration of the positive control compound.
   d. CMC control group: mode of administration missing.
   e. Details on the rat sacrificial procedure and blood sampling are not provided (required for considerations in terms of animal ethics and possible drug interaction if anaesthetics are used).
   f. “the dosage of 30 mg/kg ….”: is this sentence referred to the TAE dose?

3. Microsomal CYPs activity detection
   a. “0/100/2.5/20/5 µM PHE/TOL/DEXM/CHL/MDZ”: please specify the purpose of these substrates (please correlate them to respective CYPs monitored)
   b. Information on the LC-MS/MS setup/condition are not available
   c. Information on the methods and results for the recovery (extraction efficiency) of analytes are not available.
   d. Information related to the translation LC-MS/MS data to enzyme activity are not provided.

4. RT-PCR
   a. The amount of tissue subjected for processing and the method/procedure of tissue processing (particularly the intestine) was not provided.

5. Statistical analysis:
   a. Please specify post-hoc test employed for one-way ANOVA.

Discussion section:
CYP2E1 induction associated with TAE toxicity

Paragraph 4:
“The liver injury caused by YHS may thus have resulted from the induction of the drug metabolic enzyme CYP2E1 by long-term administration of YHS or from its metabolism by CYP2E1 to a toxic metabolite.”: please provide examples for the “toxic metabolite”

Induction ability of TAE on CYP3A1 in rats

As mentioned previously, rat may not be a good model of liver microsomal metabolism dependent on CYP3A4. The current discussion on the elevation of CYP3A1 and the indication on the risk of drug-drug interaction will be meaningful if the author can provide some examples of the clinically important drugs that are metabolised by both the rat CYP3A1 and human CYP3A4 (with relevant references), or provide references on the CYP3A1 metabolism of drug examples mentioned in the discussion.

Minor Essential Revisions

Method section:

7. RT-PCR
   a. Possible typo error in line 1: “RAN”

Discretionary Revisions

Discussion section:

The influence of TAE on other CYPs

Rats CYP2C enzymes, which is the most abundant CYP subfamily of rat liver has been previously suggested to have a role of human CYP3A enzyme. Since the current results showed that TAE only induces CYP2C11 at high concentration, the author may want to consider to re-examine the indication of the current results, particularly on its correlation to the activity of the human CYP3A enzyme.

Reference:


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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests