Author's response to reviews

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

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Author's response to reviews: see over
Subject: MS: 1686274756122430
Title: Up-regulation on Cytochromes P450 in Rat Mediated by Total Alkaloid Extract from *Corydalis yanhusuo*

Dear Sir,

We are extremely thankful for the invaluable comments we have received from reviewers who have generously shared their precious time and professional expertise to help us improve this paper. We are even more grateful to the reviewer for his insightful scientific suggestions to help make the manuscript more suitable for publication in *BMC Complementary and Alternative Medicine*. We have therefore revised the manuscript in keeping with the reviewers’ comments below. Taking all your comments into account, we have revised the manuscript accordingly and labeled them in red color. Our responses to each comment are given one after the other as raised by the reviewers.

Once again thanks for your consideration.

Yours sincerely,
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Reviewer: Shingen Misaka

Comments:

1. Pg 5, ln 25 and 27: Please remove “berberine” and “5.80%”
Response: thanks for your advice. We have already corrected this mistake.

P5 as follow:

The major alkaloids in YHS extract were quantified, and HPLC (Fig. 1) showed that the contents (w/w) of protopine, allocryptopin, dehydrocorydaline, tetrahydropalmatine, corydaline, tetrahydroberberine, and glaucine were 2.52%, 1.67%, 0.34%, 3.58%, 3.0%, 0.22%, and 0.66%, respectively.

2. Pg 7, ln 14. and Table 1: The internal standard “Kamaxiping” may be Carbamazepine? as stated in pg 7, ln 24.
Response: thanks for your advice. We have already corrected this mistake.

3. Pg 7, ln 24. (change to): The extraction recoveries of PAR, OHTOL, DEXP, OHCHL and OHMDZ were 86.3±2.0%, … and 90.8±1.9%, respectively.
Response: thanks for your advice. We have already corrected this mistake.

P7 as follow:

The extraction recoveries of PAR, OHTOL, DEXP, OHCHL and OHMDZ were 86.3±2.0%, 92.1 ± 1.6%, 87.2 ± 1.3%, 93.3 ± 2.0%, and 90.8 ± 1.9%, respectively.

4. Statistical analysis-Considering that this study was originally designed for the multiple comparison (5 groups), a repeat of unpaired t-test is inappropriate. The obtained data should be analyzed by one-way ANOVA and a post-hoc test such as Dunnet's or Tukey's test.
Response: thanks for your advice.

In the current study, the TAE-treated groups and positive control group were compared with the control group, so independent-sample t-test was used to analyze the differences between the enzyme activities, gene expression values with control values by SPSS 13.0.
5. Pg 12, ln 2, pg 13, ln 21. and pg 13, ln 28: “…substrate (s), inhibitor (s), and (or) inducer (s).” The words “inhibitors, and inducers” should be deleted, because this study was designed to investigate the possible interactions of YHS with metabolisms of substrates by CYPs, not the interactions between YHS and CYP inhibitors or inducers.

Response: thanks for your advice. We have already corrected this mistake.

P12-14 as follow:

Drug-drug interactions are of concern when low-dosage TAE from YHS as well as substrates of CYP2E1 are administered.

And TAE did not affect CYP2D1 mRNA level and activity in three doses, the finding suggests that the use of products containing YHS may be considered safe when co-administration with CYP2D1 substrates.

The results of the present dose–response study in rats suggest that potential CYP2E1 and CYP3A drug-drug interactions are unlikely at clinical dosages of TAE, but need to be considered when high dosages of TAE or TAE-containing products are co-administered with substrates of CYP1A2 or CYP2C11.

6. Conclusion – The third sentence “The results of the present dose-response study …” is hard to understand, and thus should be divided into two separate sentences.

Response: thanks for your advice. We have already corrected this mistake.

P13-14 as follow:

The results of the present dose-response study in rats suggest that potential CYP2E1 and CYP3A drug-drug interactions are unlikely at clinical dosages of TAE, but need to be considered when high dosages of TAE or TAE-containing products are co-administered with substrates of CYP1A2 or CYP2C11.
Reviewer: LIK VOON KIEW

Comments:

1.a. CMC control group: please provide information on the amount of CMC administered

Response: thanks for your advice. We have added the amount of administration of CMC on P6.

1.b.i. What are the dose of urethane administered in the current study?

Response: thanks for your advice. We have added the dose of urethane administered in the current study on P6.

1.b.ii. Does the urethane treatment contributes to the increase of CYP2E1 and CYP1A2 activities observed in the current study? Please further elaborate / explain your answers. And 1.b. iii. Does the urethane treatment affects the accuracy of the observed results of CYP3A1 activity in the current study? Please further elaborate / explain your answers.

Response: Thanks for you give the information that the influence of urethane on rat cytochrome P450 in vivo. We know that the rats received a single dose of 1.2 g/kg via intraperitoneal (i.p.) injection of urethane (40 % w/v solution in normal saline 0.9% NaCl) (Meneguz, A. et al. (1999)). As shown in Table 1 when urethane-treated animals started to lose the righting reflex (about 15 minutes) they showed no change in the basal total content of cytochrome P450. In contrast, changes in CYP IA (+63 % EROD activity, p < 0.01), CYP 2E1 (+15 % aniline hydroxylase activity, not reaching statistical significance), and CYP 3A (- 40 %, p < 0. 01) were observed after urethane. Fig.1 have shown that the rats were killed at 90 minutes after urethane administration, CYP2E1 activity was significantly increased ( p < 0.01). In the current study, the rats were quickly anaesthetized with urethane (140 mg/kg, 20% w/v solution in 0.9% NaCl) and then sacrificed by exsanguination of aorta abdominalis. In the current study, the dosage of urethane (140 mg/kg, 20% w/v solution in 0.9% NaCl) is smaller than the dosage of urethane of (1.2 g/kg, 40% w/v solution in 0.9% NaCl) in the reference.
In the current study, the rats were quickly anaesthetized with urethane (about 3 minutes or less) and then sacrificed by exsanguination of aorta abdominalis. We can insure that CYP2E1 activity in rat was not increased by urethane. Because the smaller dosage of urethane and the shorter duration of action on the rat liver, CYP3A1 and CYP1A2 activities may not be effected by urethane. Furthermore, we analyze the differences between the enzyme activities values with control values. So urethane treatment does not contribute to effect on CYP2E1, CYP1A2 and CYP3A1 activity in the current study.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total CYP</th>
<th>EROD (CYP 1A)</th>
<th>PROD (CYP 2B)</th>
<th>Aniline Hydroxylase (CYP 2E1)</th>
<th>Ethylmorphine-N-demethylase (CYP 3A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urethane</td>
<td>0.68 ± 0.05</td>
<td>*15.6 ± 1.8</td>
<td>1.85 ± 0.29</td>
<td>0.75 ± 0.05</td>
<td>*8.2 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>0.71 ± 0.04</td>
<td>9.5 ± 2.1</td>
<td>1.7 ± 0.3</td>
<td>0.65 ± 0.07</td>
<td>12.85 ± 0.2</td>
</tr>
<tr>
<td>Ketamine</td>
<td>0.70 ± 0.05</td>
<td>9.8 ± 1.9</td>
<td>2.0 ± 0.35</td>
<td>0.68 ± 0.005</td>
<td>*9.0 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>0.67 ± 0.06</td>
<td>10.4 ± 2.5</td>
<td>1.9 ± 0.3</td>
<td>0.71 ± 0.03</td>
<td>13.2 ± 0.18</td>
</tr>
</tbody>
</table>

Results are mean value ± SEM of six rats in each of the groups killed 15 min after treatment; CYP 1A was determined as EROD activity, CYP 2B as PROD activity, CYP 2E1 as aniline hydroxylase activity and CYP 3A as ethylmorphine-N-demethylase activity.

Units for total CYP are nmol/mg protein, for CYP 1A and CYP 2B are pmol resorufin/min/mg protein for CYP 2E1 are nmol PAP/min/mg protein and for CYP 3A nmol HCHO/min/mg protein.

* p < 0.01 compared with control rats.

Fig. 1. Time course effects of urethane on rat liver cytochrome P450s activities in rats. Data points represent means ± SE of 6 rats per group and are expressed as percentage of control values, that for EROD (CYP 1A) 10.6 ± 1.7 pmol resorufin/min/mg protein, for aniline hydroxylase (CYP 2E1) 0.63 ± 0.08 nmol PAP/min/mg protein and for ethylmorphine-N-demethylase (CYP 3A) 14 ± 0.4 nmol HCHO/min/mg protein. * p < 0.01 versus control value.
2.a. Only rat liver was mentioned for microsome isolation. What about the rest of the tissue collected?

Response: thanks for your advice. Cytochromes P450 (CYPs) is a group of hemoproteins that play a central role in the oxidative metabolism (phase I) of clinically-used drugs and other xenobiotics. CYPs can be found in virtually all organs notably the liver, intestine, skin, nasal epithelia, lung and kidney, but also in testis, brain etc. However, the liver (300 pmol of total CYPs/mg microsomal protein) and the intestinal epithelia (~ 20 pmol of total CYPs/mg microsomal protein) are the predominant sites for P450-mediated drug elimination, while the other tissues contribute to a much smaller extent to drug elimination (Martignoni M, et al. (2006)). In many researches, the rat liver microsome were isolated for study enzyme activity (Ying Guo, et al. (2011); Chang Zhou, et al. (2011)). So the rest of the tissue collected were used for study gene expression.

2.b. How much tissue was used for the preparation of microsomal protein isolates?
Response: thanks for your advice. We have added the amount of tissue which was used for the preparation of microsomal protein isolates on P7.

3.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.

Response: thanks for your advice. We have improved clarity the sentence “when these exceed the cellular detoxification systems”.

P11 as follow:

CYP2E1-mediated metabolism generates reactive oxygen species, such as oxygen and hydroxyl radicals, when these exceed the cellular detoxification systems, it results in oxidative stress with its various pathologic consequences.

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”.

Response: thanks for your advice. We have given further explanation on this sentence.

P12 as follow:

Consumption of YHS or YHS-containing products with the substrates of CYP3A should be taken more attention because of the possibility of drug-drug interactions.