Author's response to reviews

Title: 3,4-Dihydroxytoluene, a Metabolite of Rutin, Inhibits Inflammatory Responses in Lipopolysaccharide-Activated Macrophages by Reducing the Activation of NF-kappaB Signaling

Authors:

Kang-Yi Su (suky@ntu.edu.tw)
Chao Yuan Yu (kfhua@niu.edu.tw)
Ya-Ping Chen (kii816@yahoo.com.tw)
Kuo-Feng Hua (kuofenghua@gmail.com)
Yi-Lin Sophia Chen (a221865880@yahoo.com.tw)

Version: 7 Date: 2 January 2014

Author's response to reviews:

Dear editor:

We very thank reviewer’s valuable comments and suggestion and the chance to publish this work on the journal. We carefully checked these issues and responded point by point as followed. We very appreciate your kindly reconsideration for publication.

Editor

We note that the plant material used in your study was a gift from Biopure Biotechnology. Could we ask you to confirm whether this company played any other role in the funding, design, implementation or analysis of your study? If so, then we would ask you to please include this information in your Competing Interests section.

Response:

We thank the editor’s careful suggestion. Actually, the plant material was only a gift from Biopure Biotechnology without any commercial funding. All experiments, design, analysis and interpretation were done in academic research. We had declared “All of the authors declared that they have no competing interests.” in the title page. Thank you for your kind reminding.

Reviewer 1:

The manuscript still contains a myriad of errors and should be corrected by a native English speaker prior to be acceptable for publication.

Exemples below:

Results and discussion

-To test the inhibitory effect of rutin metabolites on inflammation, we first measured the nitric oxide (NO) production, an inflammation marker, on LPS-simulated RAW 264.7 cells pretreated with rutin or its metabolites including
DHT, DHPAA, HPAA and HVA. The result indicates that DHT had a powerful inhibitory effect at 10mM on NO product...

-In the sentence: “Since NO production can be catalyzed by inducible NO synthase (iNOS), we further reduces iNOS expression in a dose dependent manner.” There must be some words missing: Since NO production can be catalyzed by inducible NO synthase (iNOS), we further investigated the effect of DHT on iNOS expression. The result showed that DHT can significantly reduce iNOS expression in a dose dependent manner.

- The results indicate that LPS can indeed induce MAPK signaling, however, DHT had no effect on this signaling (Figure 4). Indeed, ....

- According to this signaling, we found that DHT can significantly reduce the phosphorylation level of I#B-a in LPS-stimulated RAW 264.7 cells.

- These results indicate that DHT treatment attenuate the activation of LPS-stimulated NF#B signaling cascade in macrophages.

- Taken together, our results show that the metabolites of rutin, DHT, can efficiently inhibit LPS-stimulated inflammatory response via I#B-NF#B signaling.

- Our results suggeste that the reduction of iNOS and COX-2 may be the consequence of NF#B inactivation.

Response:
Thank the reviewer’s comments and suggestion. We are very sorry for the language issue. We have correct it as following point by point in order to more readable by readers. Please check.

1. The paragraph “To test the inhibitory effect of rutin metabolites on inflammation, we first measured the nitric oxide (NO) production, an inflammation marker, on LPS-simulated RAW 264.7 cells pretreated with rutin or its metabolites including DHT, DHPAA, HPAA and HVA. The result indicates that DHT had a powerful inhibitory effect at 10mM on NO product...” had been corrected to “To test whether rutin and its’ metabolites can efficiently inhibit inflammation, we measured nitric oxide (NO) production, an inflammation marker, in LPS-stimulated RAW 264.7 cells in the present of these compounds. The result indicated 10#M DHT had the most significant inhibitory effect on NO production when compared with other metabolites (Figure 1a).” . (Page 8, line 21-25)

2. We thank the reviewer’s kind help. We had corrected it as your suggestion: Since NO production can be catalyzed by inducible NO synthase (iNOS), we further investigated the effect of DHT on iNOS expression. The result showed that DHT can significantly reduce iNOS expression in a dose dependent manner. (Page 9, line 14-16)

3. The sentences “The results indicate that LPS can indeed induce MAPK signaling, however, DHT had no effect on this signaling (Figure 4). Indeed, .....” had been corrected as “The results indicated that DHT had no effect on neither LPS mediated MAPK signaling (Figure 4) nor ERK1/2, p38 or JNK1/2 signaling.” (Page 9, line 21-23)
4. The sentence “According to this signaling, we found that DHT can significantly reduce the phosphorylation level of I#B-a in LPS-stimulated RAW 264.7 cells.” had been corrected as “We found that DHT can significantly reduce the phosphorylation of I#B-a in RAW 264.7 cells after LPS treatment (Figure 5(a)).”. (Page 10, line 4-6)

5. The sentence “These results indicate that DHT treatment attenuate the activation of LPS-stimulated NF-#B signaling cascade in macrophages.” had been corrected as “These results indicated that DHT at least can attenuate NF-#B signaling activation in macrophages after LPS treatment.” (Page 10, line 7-8)

6. The sentence “Taken together, our results show that the metabolites of rutin, DHT, can efficiently inhibit LPS-stimulated inflammatory response via I#B-NF#B signaling.” had been corrected as “Taken together, our results showed that DHT can efficiently inhibit I#B-NF#B mediated inflammatory response. (Figure 6)”. (Page 10, line 9-10)

7. The sentence “Our results suggeste that the reduction of iNOS and COX-2 may be the consequence of NF-#B inactivation.” had been corrected as “Although the reduction of iNOS and COX-2 may be the consequence of NF-#B inactivation, other possible mechanisms involved in DHT effects can’t be excluded due to wide contribution of NF-#B.”. (Page 11, line 20-22)

Reviewer 2

This manuscript describes the anti-inflammatory activity of rutin derivates from chineese Saussurea involucrate. The authors tested these effects with several in vitro tests. They showed that the component of rutin responsible of its anti-inflammatory activity. The study is well conducted and authors had integrated almost all the modifications suggested by the reviewers. So this paper could be published and this work would be useful for person with closely related work.

Response:

We very thank the reviewer’s comments. We also appreciate the reviewer’s effect on the improvement of this manuscript.