Author's response to reviews

Title: Effects of Ginsenoside Re on LPS-induced Inflammatory Mediators in BV2 Microglial Cell

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Author's response to reviews: see over
Dear Editor-in-Chief of BMC Complementary & Alternative Medicine

Ref: MS 1053585881765365

Title: Effects of Ginsenoside Re on LPS-induced Inflammatory Mediators in BV2 Microglial Cell

On the behalf of my co-authors, I would like to thank you for arranging the re-review of our manuscript and for your invitation to submit a revised version. We appreciate the effort of the reviewers, and believe that their constructive suggestions have resulted in a stronger manuscript for readers of the BMC Complementary & Alternative Medicine.

Yours faithfully,

Eun Jin Yang, PhD
Dear Reviewer,

RE: Effects of Ginsenoside Re on LPS-induced Inflammatory Mediators in BV2 Microglial Cell (MS: 1053585881765365)

We appreciate the minor comments from the reviewer regarding our manuscripts. According to his (her) suggestions, my colleagues and I have made the appropriate changes in a point by point manner. The responses are detailed as follows:

Reviewer(s)' Comments to Author:

**Comments of Reviewer #1:**

1. This article reported a study on the effects of ginsenoside Re on LPS-induced inflammatory mediators in BV2 microglial cells. After reading the article I found that this is a simple and less novel study providing less new information to the audience except with another cell line. Similar but more detailed study has been published by Wu et al. (International Immunopharmacology 7 (2007) 313–320) which shows G-Re, at different doses inhibits LPS-induced pro-inflammatory factors release and more detailed mechanisms of action have been evaluated than what has been done by the present article.

   ➔ Answers and revised points

We agree with the reviewer’s comment that the previous study by Wu et al. had a similar focus, in that they showed the effect of G-Re on LPS-induced N9 microglial cells. However, they illustrated the inhibitory effect of LPS-induced NO formation and TNF-α production by G-Re via inhibition of the NF-κB pathway. In the case of our study, we showed that the
reducing effect of LPS-induced proinflammatory production by G-Re was caused by the inhibition of p38MAPK and activated caspase-3 expression leading to cell death, as shown in Fig. 1C and Fig. 2A.

2. The present study did not well cite similar studies, especially ignored the results published by Wu et al. about G-Re’s effects either in the Introduction or in the Discussion that mislead the novelty of the study. For example, in Introduction “Several studies have reported the neuroprotective effects of Rg1 or its metabolites, but not of Re.” In Discussion “Although some evidence from this study showed that LPS-induced JNK activation was attenuated by ginsenoside Rb1 [23], ginsenoside Rg1 [24], and ginsenoside Rd [25], these compounds were not consistent with our G-Re compound or the use of different cell lines.”

**Answers and revised points**

We agree with the reviewer’s comments. We rewrote the introduction and discussion, and cited the paper by Wu et al. in these sections of our manuscript.

3. Only one dose of G-Re was used for the most important parameters in the present study. I wonder whether this is a threshold or a maximal dose that produced these effects.

**Answers and revised points**

We treated with 0.5–100 μg/ml of G-Re to determine cell toxicity and found that G-Re treatment did not induce cell toxicity in BV2 cells, in accordance with a previous paper. In addition, we found that treatment with 2 μg/ml of G-Re inhibited LPS-induced cell death, as well as the activation of p38MAPK and activated caspase-3 by LPS treatment in BV2 cells, as shown in Fig 1C and 2A. However, we expect that a high dose (more than 2 μg/ml) of G-Re could attenuate LPS-induced cell death and change the expression of other proteins to a greater extent than in the present study. According to Wu et al.’s paper (*International Immunopharmacology*, 2007, 313-320), they used 0.1–100 μM of G-Re. However, the effect of high-dose G-Re seems to have been similar to that of the low dose in nitric oxide or TNF-α
production. In addition, the only high dose of G-Re treatment inhibited the pJNK and pIκBα expression. Therefore, we suggest that the effect of G-Re treatment will be increased at a high dose, depending on the target proteins and cell lines.

**Comments of Reviewer #2:**

In this paper the authors provide evidence that G-Re (ginsenoside-Re) attenuates LPS-induced microglial toxicity and pro-inflammatory activation. More specifically, they demonstrated that G-Re prevents LPS-induced microglial cell death and attenuates LPS-induced activation of p38MAPK in BV2 cells. In addition, G-Re attenuates the protein expression of LPS-induced pro-inflammatory mediators in BV2 microglial cells. These data support that p38MAPK is important for microglial cell death and pro-inflammatory cytokine up-regulation in response to LPS and may be a therapeutic target for neuroinflammatory diseases, where overproduction of pro-inflammatory cytokines has been implicated in disease progression. G-Re is one promising therapeutic approach for the treatment of neuroinflammatory diseases.

The studies are well designed and performed properly, and the methods are appropriate and well described. The data are also supportive of the conclusions. Nevertheless, there are several minor concerns to be addressed:

1. In the legend of Fig. 1, the last sentence, 'The values shown are the mean~' is not applied to (C). So, it should be move to the back of the legend of (A) and (B).

   ➔ Answers and revised points>

   According to the review’s comment, we moved the last sentence, 'The values shown are the mean~' to the back of the legend of (A) and (B) and marked the change in yellow.

2. In whole legends of Figures, the description of statistical value ‘P< number’ should be corrected to ‘p < number.

   ➔ Answers and revised points>
According to the review’s comment, we corrected statistical value to ‘p < number’ and marked the change in yellow.

3. In Figs 1, 3, and 4, they only showed the single picture of immunostaining data. For consistency, at least two pictures should be provided for each sample.

**Answers and revised points**

According to the review’s comment, we added one picture more for each sample in figures 1, 3, and 4.

**Editorial requests and reminders:**

1. Abstract

Please ensure that your abstract is in accordance to the guidelines for authors <http://www.biomedcentral.com/info/ifora/abstracts>. Please make sure it is identical to the one in the submission system.

**Answers and revised points**

According to the editorial request, we corrected abstract in accordance to the guidelines for authors and marked the change in yellow.

2. Structure

Please check the instructions for authors on the journal website to ensure that your manuscript follows the correct structure for this journal and article type.

**Answers and revised points**

According to the editorial request, we reorganized our manuscript in accordance to the guidelines for this journal and article type.