Author’s response to reviews

Title: Chlorogenic Acid Protects PC12 Cells Against Corticosterone-induced Neurotoxicity Related to Inhibition of Autophagy and Apoptosis

Authors:

Xiaowen Shi (xiaowenshi1990@163.com)
Nian Zhou (kyky654321@163.com)
Jieyi Cheng (13611738323@163.com)
Xunlong Shi (xunlongshi@fudan.edu.cn)
Hai Huang (hhai3552@sina.cn)
Mingmei Zhou (zhoumm368@163.com)
Haiyan Zhu (haiyanzhu@fudan.edu.cn)

Version: 2 Date: 22 Aug 2019

Author’s response to reviews:

Dear Dr. Graeme Sills,

Thank you again for your patient and professional guidance to us. We have revised the manuscript again to fully follow the reviewers’ comments, and the amendments are highlighted in the manuscript in revised mode. Below this letter is our explanation on revision according to the comments of the reviewers.

We hope that this further revised version of the manuscript is now acceptable for publication in your journal.

Thank you for your kindly help again, and I am looking forward to hearing from you soon.

With best wishes,

Yours sincerely,

Mingmei Zhou, MD, PhD, Associate Professor

Corresponding author
The points need to be further revised raised by Reviewer #2 (Dr David Moranta):

1. Major point 2: re-blotting and housekeeping proteins

Thank you very much for your guidance. Based on your suggestions, information about re-blotting and housekeeping proteins was added in the manuscript, see “2.6 Western blot”, line 159-161, page 6

2. Major point 3: scientific basis of call viability assay

Thank you for your comments. In this experiment, the activity of mitochondrial succinate dehydrogenase in living cells was tested after 24 hours of administration by MTT assay to reflect the effect of the drug on cell viability. And the literature [31] claims that the MTT method is a sensitive detection method with linearity up to 106 cells/well, and subtle changes in metabolic activities can lead to large changes in MTT. Therefore, we used the MTT method to detect cell viability. Related information has been added to the manuscript, see “2.3 Cell viability assay”, line 126-130, page 5.

3. Major point 4: comment on time-course of changes in LC3II expression

Thank you for your comments. In the results, Fig. 1B, C suggest that the LC3-II / LC3-I ratio increases along with the time and is related to the degree of cell damage. The corresponding description has been added to the manuscript, see the section of “Results”, line 176-177, page 6.

4. Major point 5: comment on changes in PARP density

Thanks the advice from the editor. In the early stages of apoptosis, PARP is cleaved by caspase. Caspase-3 cleaves the unactivated form of PARP protein into two fragments, which is considered to be a characteristic feature of apoptosis. Both Fig. 5B and Fig. 2D showed a tendency for CGA to inhibit apoptosis, as well as significantly inhibition of the expression of C-PARP, and there was no significant inhibitory activity against uncleaved RARP. Therefore, there was no significant change in the concentration of PARP. Instructions have been added to the manuscript, see the section of “Discussion”, line 256-261, page 9.

5. Major point 6: explain how densitometric analysis relates to WB images

Thanks the advice from the editor. In order to make the experimental results more reliable, independently WB test were repeated 3 times (n=3), and the grayscale data analysis is an average
analysis of three replicates. Therefore, the graphs and data are not completely consistent. The related explanation has been added in the manuscript, see section “2.6 Western blot”, line 161-162, page 6

6. Major point 8: explain the choice of 25μg CGA

Thanks for your suggestions. In the pretest, we tested 4 concentrations, and the results showed that 25 μg/ml CGA could exert neuroprotective effects on CORT-induced nerve injury, such as significantly increasing cell viability compared with the model group, significantly reducing the amount of C-PARP and the conversion rate of LC3I to LC3II. However, the in vitro activity test of traditional Chinese medicine often has HOOK effect. Therefore, we chose a medium dose of 25 μg/ml with strong activity. We have added corresponding explanations in the manuscript. For details, see the section of “Results”, line 226-232, page 8.

7. Minor point 1: comment on this shortcoming in the discussion section

Thank you for your valuable comments. Some related discussions and explanations have been added, see the section of “Discussion”, line 290-292, page 10.