Author's response to reviews

Title: epigallocatechin-3-gallate suppresses 1-methyl-4-phenyl-pyridine ion-induced damage by oxidative stress in PC12 cells via the SIRT1/PGC-1alpha signaling pathway

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Author's response to reviews:

To Reviewer: Beisha Tang

1. Why are the methods to study the expression of PGC-1#, SIRT1, SOD1 and GPX1 not the same?

PGC-1# and SIRT1 are multifunctional regulatory proteins. PGC-1# and SIRT1 protein expression was detected by western blot in our paper. SOD1 and GPX1 are typical antioxidant enzymes. Deacetylation of PGC-1# increases transcriptional activity and the expression of detoxification enzymes. To detect gene expression at the transcriptional level, the mRNA expression levels of SOD1 and GPX1 were detected by real-time PCR.

2. From the paper, it has not enough proof to draw the conclusion that EGCG suppresses MPP+-induced damage of oxidative stress in PC12 cell through SIRT1/PGC-1# signaling pathway. It will be more powerful if experiments can confirm EGCG loss the role when the expression of SIRT1/PGC-1# has no change.

We were able to draw the conclusion that the SIRT1/PGC-1# pathway is one of the mechanisms of EGCG acting on MPP+-induced injury of PC12 cells. We are now exploring the mechanism of EGCG using the specific SIRT1 inhibitor, sirtinol and siRNA-PGC-1#.

To Reviewer: Hirokazu Hara

1. Figs. 2 and 3: The authors provide no information about the time of treatment of cells with EGCG and/or MPP+. The reviewer thinks the treatment period is an
important factor to understand the relationship between SIRT1/PGC-1# pathway and expression of antioxidant genes. Therefore, time course studies should be performed.

In preliminary experiments, we used different concentrations of MPP+ at different time points (3, 6, 12, 24 and 72 h). We found a significant effect of MPP+ on PC12 cell viability after 6 h. The most pronounced effect at 24 and 48 h. Therefore, we choose 24 h as an observation time point.

2: EGCG had protective effect against MPP+-induced cell death. However, treatment with EGCG alone failed to induce protein expression of SIRT1 and PGC-1#, whereas co-treatment with EGCG and MPP+-induced SIRT1 and PGC-1# expression. The reason why MPP+ enhances expression of these proteins should be discussed.

EGCG is an antioxidant. During MPP+-induced oxidative stress, EGCG significantly increased the levels of SIRT1 and PGC-1# to show a strong antioxidant effect. During non-oxidative stress, EGCG is not sufficient to induce activation of the SIRT1 /PGC-1# pathway.

3: Fig. 3 shows that EGCG induces antioxidant genes such as SOD1 and GPX1. Therefore, the authors mention in the text that reactive oxygen radicals scavenging effects of EGCG contribute to EGCG protection against MPP+ toxicity. If so, did the authors check whether treatment with EGCG decrease levels of intracellular oxygen radicals after MPP+ exposure?

To measure ROS production in PC12 cells after MPP+ exposure, we used a DCFH-DA assay, described as follows. DCFH-DA is a fluorescent dye that crosses the cell membrane and is enzymatically hydrolyzed by intracellular esterases to non-fluorescent DCFH. The result showed that EGCG attenuated the generation of intracellular ROS after MPP+-induced oxidative stress (see Fig. 2).

4: The authors argue that expression of the antioxidant genes is mediated via the SIRT1/PGC-1# pathway in EGCG treated PC12 cells. However, it is not clear whether EGCG-induced activation of the SIRT1/PGC-1# pathway leads to induction of the antioxidant genes. Therefore, this conclusion is suggestive but not definitive. Does resveratrol, an activator of SIRT1, or sirtinol, an inhibitor or SIRT1, influence EGCG protection or expression antioxidant genes?

We could draw the conclusion that SIRT1/PGC-1# pathway is one of the mechanisms of EGCG acting on MPP+-induced injury of PC12 cells. We are now exploring the mechanism of EGCG using the specific SIRT1 inhibitor, sirtinol and specific SIRT1 activator, SRT1720.