

## OPEN PEER REVIEW REPORT 1

**Name of journal:** Neural Regeneration Research

**Manuscript NO:** NRR-D-19-00640

**Title:** Mitochondrial acid-5 promotes the survival of mouse microglial BV-2 cells under LPS-induced inflammation via MFN2-associated mitophagy

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**Reviewer's country:** Portugal

### COMMENTS TO AUTHORS

This is an interesting manuscript that explores a possible approach to protect microglia against inflammation-induced apoptosis. The authors demonstrated that after LPS-induced inflammation MA-5 treatment prevents BV-2 cells apoptosis and promote their migration, being these beneficial effects associated to MFN2-related mitophagy. The regulation of microglial activity and the prevention of apoptosis after an inflammatory stimulus are seen as an interesting target in several pathological conditions that affect the brain. The importance of microglial cells in brain homeostasis and their protective role on several neurodegenerative disorders (e.g. ischemic injury, Alzheimer's disease and Parkinson's disease) is gaining strength, therefore any approach that protect and/or potentiate the microglial activity should be carefully analyzed. There are several issues needing attention to improve the manuscript quality before publication. These are listed individually below.

Page 3, line 9 to 11: "Stimulation of microglial cells with LPS has been shown to induce the production of neuroinflammatory cytokines and contribute to neuronal degeneration in vivo (Dulla et al., 2016)."

Comment: How? What are the mechanisms that are associated with this neuronal degeneration?

Page 4, line 9 and 10:

Comment: There is no link between the second and third paragraph.

Page 6, line 1:

Comment: The abbreviation "FBS" is not described in the text, is this "Fetal bovine serum"? Where was purchased? It was previously inactivated?

Page 6, line 12:

Comment: I recommend replacing the title "MTT assay and TUNEL detection" for "Cell viability assessment".

Page 15, line 5:

Comment: I suggest to replace the sentence "MA-5 alleviated the apoptosis of BV-2 cells induced by LPS." for "...MA-5 reduced the LPS-induced apoptosis on BV-2 cells."

In the literature there is any data regarding the beneficial effects of MA-5 on microglial cells after an inflammatory stimulus? Could these data be discussed?

Page 16, line 11 and 12:

Comment: The authors report that "The present study demonstrated that MA-5 enhances mitophagy via MFN-2." Are there any other cellular signaling pathways that could be involved in the beneficial effects induced by MA-5 after an inflammatory stimulus? Could these data be discussed?



Comment: Besides microglia, there is any other cellular population present on the brain where a beneficial effect of MA-5 has already been demonstrated after an inflammatory stimulus? Would it be possible to look at MA-5 as an integrative approach that has the ability to induce beneficial effects in multiple cell populations? Could these data be discussed?

Comment: Is there any information about the impact of MA-5 on astrocytes?

Figure 1 legend:

Comment: There are several abbreviations that are not described on the manuscript, such as: "FBS", "MTT", "PBS", "SDS-PAGE", "PVDF", "TBS", "ECL";

Comment: There are also some materials with catalog numbers while others are not described. I suggest standardizing the material description.

Comment: On the text there are also several symbols which I suggest to be replaced by the appropriate words, such as: "~" (page 7, line 14), ">" (page 8, line 1) and "#" (page 9 line 22).