

Peer Review File

Article information: <https://dx.doi.org/10.21037/atm-21-730>

Reviewer A

Comments: All experimental results in this manuscript are very not clear. They are very rough. Every experiment is not able to support Authors' hypothesis. Western blotting absolutely needs improvement. I cannot see any difference in Immunofluorescence results. Labeling is also not clear. English writing also needs to be improved.

Overall, I think this study still needs to be cooked and needs to be re-organized.

Reply: Because Figures submitted were low-resolution at last time, so all experimental results in this manuscript look like not clear. In the revised manuscript, we used the high-resolution Figures including Western Blotting and Immunofluorescence results. Labeling in the Immunofluorescence Figures were thickened and could be observed clearly. We have revised hypothesis in the discussion.

Changes in the text: Fig.3, Fig.4, and Fig.6 were used high-resolution Figures instead of low-resolution Figures. We have revised hypothesis in the discussion(see discussion, page 24-28).

Reviewer B

Pyrroloquinoline quinone (PQQ) is a novel stimulator of mitochondrial biogenesis and cellular energy metabolism. The Authors used various techniques to establish the role of PQQ in the autophagy of HAPI microglial cells. Although the obtained results are interesting and investigate novel mechanisms, there are some major issues that need to be considered:

Comment 1: For many bio-factors, the ability of given compounds to form adducts is an issue. The inability to assay total amounts of adducts plus the putatively active component(s) is another. PQQ is converted to imidazole derivatives, such as imidazole pyrroloquinoline or protein-PQQ adducts. It has been addressed in recent paper (10.3390/ijms21218382) that it is hard to measure how much free PQQ is available as a product of the equilibrium between PQQ and its potential adducts. It

would be worth mentioning that the observed effects are likely the sum of both free PQQ and its derivatives actions.

Reply: According to the comment, we mentioned that the observed effects are likely the sum of both free PQQ and its derivatives actions.

Changes in the text: We have modified our text as advised (see Page 25, line 6-8).

Comment 2: The methods description seems detailed, describing procedures step by step, although the term ‘good condition’ about culture is not convincing. The passage is usually done based on the confluency. What would be the symptoms of cells being in ‘bad condition’?

Reply: According to the comment, the term ‘good condition’ about culture should be revised as the growth status of cells is confluency.

Changes in the text: We have modified our text as advised (see Page 9, line 19).

Comment 3: The bands in Western Blot are cutted out so narrowly, especially for TNFR and LAMP2. The larger area would help to confirm the specificity of antibodies used.

Reply: According to the comment, we have used the original Western Blot Figures of TNFR and LAMP2 whose area are larger. Because Western Blot was done by using protein Marker, so we could judge which band is the objective band according to the molecular mass size.

Changes in the text: We have modified our text as advised (see Fig.4C, Fig.6A, and Fig.6 A).

Comment 4: The description of the results 3.8 is not understandable and requires rewriting.

Reply: We have rewritten the results 3.8.

Changes in the text: We have modified our text as advised (see Page 24, line 1-10).

Comment 5: The discussion is quite chaotic and it is hard to notice the Authors conclusions.

Reply: The discussion has been revised according to the results of this study, according to the opinion from the references. At the end of the discussion, we draw a conclusion.

Changes in the text: We have modified our discussion as advised (see Page 24-28).

Minor revisions:

Comment 1: The title should be modified, e.g., Effect of PQQ on lipopolysaccharide-induced autophagy in HAPI microglia cells

Reply: According to the comment, we have modified the title as “Effect of PQQ on lipopolysaccharide-induced autophagy in HAPI microglia cells”.

Changes in the text: We have modified our text as advised (see Page i, line 3-4)

Comment 2: The Authors have strange manner of describing methods, rather as an instruction than the information what has been done. These two styles are mixed for instance in 2.10 section.

Reply: According to the comment, we have revised the description about methods.

Changes in the text: We have modified our text as advised (see Page 15, line 11-17)

Comment 3: The text needs grammar and spellcheck revisions, for instance page 3 line 28, page 9 line 8 (‘diviede’), page 10 lines 25-26, page 11 line 3, page 9 line 20 (there is something missing in the sentence), page 11 line 25, page 12 lines 1-2 (not precisely specified what group and protein Authors describe), page 12 line 20, page 13 lines 11-12, page 13 lines 31-32, page 15 line 32

Reply: According to the comment, we have revised the corresponding contents in the text.

Changes in the text: We have modified our text as advised in blue font word.

Comment 4: Page 3 line 17, do authors mean ‘autolysosome’ instead of ‘autophagosome’

Reply: Autolysosomes are correct. Autophagosomes are wrong.

Changes in the text: We have modified our text as advised (see Page5, line 17)

Comment 5: Some abbreviations are not defined in the text, e.g., ATG3, SQSTM1/P62

Reply: We have checked all abbreviations from the fulltext and add some abbreviations that are not defined in the old text.

Changes in the text: We have modified our text as advised (see Page 37, line 9-11)