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Cytotoxicity of mitochondrial Complex I inhibitor rotenone: a complex interplay of cell death pathways

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Reviewer 2

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*Only major points from review and responses included.

Reviewer 2

Trypan Blue assay: What was the minimum number of cells counted per analysis?

Authors

The automated counter provides the number of cells / µL and the percentages of live and dead cells. However, we routinely cross-checked the Trypan blue assay by manual counting by two independent observers; each person counted 250 to 300 cells separately for each assay. This is now mentioned in the revised version.

Reviewer 2

The discussion needs more detail on:

- Comment on the use of undifferentiated SHSY5Y versus differentiated cells. Is there any data on ferroptosis on differentiated cells that can be considered in the discussion?

- Is ferroptosis potentially linked to the duration/concentration of treatment? Please, discuss the potential role of the length of incubation (48h) versus shorter treatments (24 hours for example) and higher concentration of Rotenone in inducing cell death processes. Would a higher dose and shorter treatment, leading to the similar % of cell death, be inducing ferroptosis and/or other cell death mechanisms?

- There is evidence that Rotenone may have Complex I independent toxicity potential. Please discuss the potential role of this characteristic in the induction of ferroptosis.

Authors

We thank the reviewer for very constructive and useful suggestions which have made our manuscript stronger. The possible effects of dose and duration of rotenone treatment, the nature of the cells including the state of differentiation on the mechanisms of cytotoxicity of rotenone have been mentioned in the Discussion from a survey of the existing literature. However, more elaborate studies are necessary to establish these effects; we will explore this aspect in our future work. Further, we have discussed some apparently Complex I – independent toxicity of rotenone such as disruption of microtubules, activation of JNK / p38 etc.