To,

The Editor

Journal of Neuroimmune Pharmacology

Subject: Submission of the revised research article

Title: S1PR2 inhibition mitigates cognitive deficit in diabetic mice by modulating microglial activation via Akt-p53-TIGAR pathway

Dear Jay Rappaport

On behalf of my co-authors, I would like to thank you for the opportunity to revise and resubmit our manuscript with reference number c1086ad0-4e94-4054-9d97-dcf7939fd06 entitled "S1PR2 inhibition mitigates cognitive deficit in diabetic mice by modulating microglial activation via Akt-p53-TIGAR pathway" for publication in your esteemed journal "Journal of Neuroimmune Pharmacology." We found the reviewers' comments to help revise the manuscript and have carefully considered and responded to each suggestion.

We have included a response to reviewers in which we address each comment the reviewers made. The reviewers' comments are numbered in our response to reviewers, and our responses follow below, in blue and italics, and are prefaced by "Author response." We are submitting the clean version of the revised manuscript. There are no changes in the main manuscript as we have thoroughly answered the reviewers’ comment in the response file.

Sincerely,

Dr. Shashi Bala Singh, FNASc, FIAN, FAMS,
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Response to Reviewer Comments
(c1086ad0-4e94-4054-9d97-dfcf7939fd06)

We are very thankful to the editor and reviewers for their deep and thorough review. We have revised our present research paper in light of their valuable suggestions and comments, and we hope our revision has improved the paper to their satisfaction level. Number-wise, answers to their specific comments/suggestions/queries are as follows.

REVIEWER REPORTS

Reviewer Comments:

Reviewer 3

The authors should be commended for addressing the issues to satisfaction.

Author's Response

Thanks for the appreciation.

Reviewer 2

The authors focus on the change in microglial phenotype that rescues cognition via the S1PR2-Akt-p53-TIGAR axis. However, there are several gaps that the authors need to demonstrate by performing different experiments. It is very difficult to interpret the involvement of specific signaling pathways in microglia in improving behavioral performance when transgenic mice are not used.

It has already been reported that the p53 transcriptional network affects microglial behavior and neuroinflammation. Thus, if the authors focus specifically on rescuing cognition via the S1PR2-Akt-p53-TIGAR axis, they will need to demonstrate that local injection of drugs into various target regions of the brain and activation of TIGAR via S1PR2 inhibition must be performed specifically in microglia.

S1PR2 inhibition attenuates cognitive deficit in diabetic mice can be justified, but the second part of the title in the article does not meet with these experiments. More evidence is needed on how microglial activation is modulated by the S1PR2-Akt-p53-TIGAR axis under in-vivo conditions. If the other factors are also involved in behavioral enhancement, then this masking of results is not novel. The authors must be specifically targeting S1PR2 in microglia and the associated signaling pathway.
Author's Response

We appreciate reviewer’s concern regarding performing additional experiments and using transgenic mice for validating our hypothesis. We apologise that these experiments could not be performed in this study. These suggestions could be incorporated in a separate study which requires additional approvals.

We agree that p53 transcriptional network has been reported to affect microglial behaviour and neuroinflammation but that has not been reported in type2 diabetes induced cognitive impairment.

We highly appreciate the reviewer’s suggestion of locally injecting JTE013 into different brain regions to prove that these areas are responsible for improving cognitive performance. As we have already mentioned regarding this point in previous response to reviewers that in our study, the behavioral tests which we have performed are mainly hippocampus and PFC-dependent. The results obtained from these tests have confirmed the involvement of PFC and hippocampus in improving cognitive performance. Numerous drugs, like metformin, telmisartan, and fingolimod, are tested for cognitive performance, administered orally or intraperitoneally to animals. The areas checked for improving cognitive performance are the hippocampus and PFC. Therefore, it is well reported in the literature to check these areas for cognitive performance. Moreover, in clinical settings, the last alternative is to directly inject the drug in the brain.


We agree that the drug would have a global effect, but that might not be responsible for cognitive performance. The behavioral and molecular data suggest mainly the involvement of
PFC and hippocampus in cognitive improvement as we had performed hippocampal and PFC-dependent memory tests, i.e., Morris Water maze, Y-maze, NORT, and passive avoidance test.

Regarding the reviewer’s point of activation of TIGAR via S1PR2 inhibition must be performed specifically in microglia. Thanks for raising this concern. It is well reported in literature that TIGAR regulates the microglial activation and pyroptosis. Studies have found that the TIGAR upregulation inhibits the inflammatory responses of microglia. P53 is the well-known upstream regulator of TIGAR. Akt and p53 signaling are linked in many diseases including neurodegeneration. Moreover, we have performed in-vitro experiments in BV2 microglial cells using palmitate and found that TIGAR was downregulated in Pal-exposed BV2 cells. S1PR2 inhibition by JTE013 in Pal-exposed BV2 cells improved TIGAR expression.

The references are mentioned below:


We totally agree that other factors can also be involved in behavioral enhancement in this study. But we have used a very specific inhibitor of S1PR2 i.e. JTE013 in our study and have reported improvement in cognition. If S1PR2 inhibition was not the major pathway involved then there would be no significant improvement in behaviour.