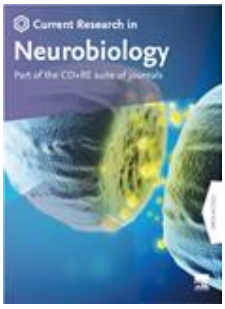
Peer Review Overview



Manuscript Title: Therapeutic Effects of TP5, a Cdk5/p25 Inhibitor, in in vitro and in vivo models of Parkinson's Disease.

Received	Oct 03, 2020
1st Decision	Dec 17, 2020
1st Revision Submitted	Feb 03, 2021
Accepted	Feb 23, 2021

1st Decision letter

Reference: CRNEUR-D-20-00011
Title: Therapeutic Effects of TP5, a Cdk5/p25 Inhibitor, in in vitro and in vivo models of Parkinson's Disease.
Journal: Current Research in Neurobiology

Dear Dr. Mishra,

Thank you for submitting your manuscript to Current Research in Neurobiology and I do apologize for the delay in getting the reviews returned to reach a decision.

The reviewers recommend reconsideration of your manuscript following major revision. I invite you to resubmit your manuscript after addressing the comments below when you are able. Please resubmit your revised manuscript when you have been able to address the points.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

Current Research in Neurobiology values your contribution and I look forward to receiving your revised manuscript.

Kind regards,

Christopher I. Petkov
Editor in Chief
Current Research in Neurobiology

Comments from Editors and Reviewers:

Reviewer #1:

The manuscript purpose is to investigate the neuroprotective and neurorestorative properties of Truncated Peptide 5 (TP5), a derivative of the p35 activator involved in Cdk5 regulation, via the inhibition of Cdk5/p25 complex function. They examined if TP5 can act as a Cdk5/p25 inhibitor against Paraquat that induces Parkinsonian like properties. The authors used SH-SY5Y culture cells and the worm C. elegans to determine the effects in the dopaminergic system. There are a few modifications that I will suggest to improve the manuscript quality and clarity

1) Please insert a reference for the microscopy section in methods. Please clarify how did you calculate the neurodegeneration % indicated in the figures that were used.
2) I have a huge concern with the % of neurodegeneration indicated in the control group. In general neurodegeneration in control groups is very close to zero in several papers. The data presented here indicated 50% of animals with neurodegeneration in dopaminergic neurons. Several kinds of stress could cause neurodegeneration in adult worms. Please consider including some explanation for this observation in the discussion section.
3) In figure 6C the asterisk did not indicate a proper neurodegeneration site. The figure is not clear and several sites in the figure look like a neurodegenerative site (as indicated by the authors). The dopaminergic neurons did not look degenerative as you could observe in other manuscripts (PLoS Genet 2010 Aug 26;6(8) : e1001084 doi: 10.1371/journal.pgen.1001084; Neurotoxicology 2020 Mar;77:105-113 doi: 10.1016/j.neuro.2020.01.003. Epub 2020 Jan 11). Did you consider to use a different strain (dat-1::mCherry) in order to improve or confirm your data? In my opinion, there are several points in the figures that could be indicated as an asterisk. Please clarify this point.
4) The figure 7 (A-D) present could be changed for better pictures. Some of them as hard to have a clear image from dopaminergic neurons.
5) I strongly suggest not use the manuscript in preparation as a reference once you have published data.

Reviewer #2:

The manuscript investigated therapeutic effects of TP5 in PD models. The truncated peptides supposed to block Cdk5/p25 complex and authors demonstrated the important role of TP5 to inhibit Cdk5/p25 activity in PD pathogenesis. However, the major concern is that it will be appreciated if representative images/data could be presented with statistical quantification in the figures 3/4/5/8. Some other concerns as the following:
1. It seems like that the synthetic peptide could penetrate plasma membrane. Does it contain any cell-penetrating peptide sequence?
2. In figure 4, the data showed the similar toxic effects of TP5 and SCRM TP in SH cells which complicated the interpretation of the data.
3. In section 3.4, the title was not specific to address at which levels of Cdk5/p25 was increased. The authors immunoprecipitated Cdk5 with specific antibodies for kinase activity assay, did TP5 also block the interaction of Cdk5 with the antibody? It would be great to show the quantity of immunoprecipitated protein by western blot.

1st Author Response Letter

Response to comments from Editors and Reviewers:

We are thankful to editors and the reviewers for spending their time in reviewing our manuscript and providing constructive criticisms. We have addressed all the concerns and believe that changes have greatly improved the quality of the manuscript.

Comments from Reviewer 1

The manuscript purpose is to investigate the neuroprotective and neurorestorative properties of Truncated Peptide 5 (TP5), a derivative of the p35 activator involved in Cdk5 regulation, via the inhibition of Cdk5/p25 complex function. They examined if TP5 can act as a Cdk5/p25 inhibitor against Paraquat that induces Parkinsonian like properties. The authors used SH-SY5Y culture cells and the worm C. elegans to determine the effects in the dopaminergic system. There are a few modifications that I will suggest to improve the manuscript quality and clarity

1) Please insert a reference for the microscopy section in methods. Please clarify how did you calculate the neurodegeneration % indicated in the figures that were used.

We have inserted the following two references for the microscopy section:

Taylor, S. K. B., Minhas, H. M., Tong, J., Selvaganapathy, P. R., Mishra, R. K., & Gupta., B. P. (2021). C. elegans electrotaxis behavior is modulated by heat shock response and unfolded protein response signaling pathways. Nature Sci *Rep*. (in press). https://doi.org/10.1038/s41598-021-82466-z

Richman, C., Rashid, S., Prashar, S., Mishra, R., Selvaganapathy, P. R., & Gupta, B. P. (2018). C. elegans MANF Homolog Is Necessary for the Protection of Dopaminergic Neurons and ER Unfolded Protein Response. Front. Neurosci., 12, 544. https://doi.org/10.3389/fnins.2018.0054

The calculations for neurodegeneration are based on the above two papers. These have been described in Methods Section 2.8.

2) I have a huge concern with the % of neurodegeneration indicated in the control group. In general neurodegeneration in control groups is very close to zero in several papers. The data presented here indicated 50% of animals with neurodegeneration in dopaminergic neurons. Several kinds of stress could cause neurodegeneration in adult worms. Please consider including some explanation for this observation in the discussion section.

We have provided an explanation for this observation in the section 2.8 Dopaminergic Neurodegeneration, second paragraph. As mentioned in our methods, we studied abnormalities in the dopaminergic neuron that include defects in cell count as well as subtle changes in neuronal projections such as blebbing, punctate pattern, deformed shape, faint appearance, and complete absence. This wide range of phenotypic scoring is likely the reason for higher degeneration in our analysis.

Please note that dopaminergic neurons are more susceptible to deterioration with age. Our model consists of using Day 3 and 5 worms to resemble a Parkinson's disease model. In the research of Yin et al. (2014), when measuring dopamine level using formaldehyde- induced fluorescence (FIF) technique, day 5 N2 worms were found to only have 71% fluorescence intensity compared to Day 1 worms that were normalized to 100%, demonstrating the effects of aging towards dopaminergic neurons. Moreover, we have shown earlier (Richman et al. 2018) that dopaminergic neurons degenerate with age.

Yin, J. A., Liu, X. J., Yuan, J., Jiang, J., & Cai, S. Q. (2014). Longevity manipulations differentially affect serotonin/dopamine level and behavioral deterioration in aging Caenorhabditis elegans. The Journal of neuroscience: the official journal of the Society for Neuroscience, 34(11), 3947–3958. https://doi.org/10.1523/JNEUROSCI.4013-13.2014

Richman, C., Rashid, S., Prashar, S., Mishra, R., Selvaganapathy, P. R., & Gupta, B. P. (2018). C. elegans MANF Homolog Is Necessary for the Protection of Dopaminergic Neurons and ER Unfolded Protein Response. Front. Neurosci., 12, 544. https://doi.org/10.3389/fnins.2018.00544

3) In figure 6C the asterisk did not indicate a proper neurodegeneration site. The figure is not clear and several sites in the figure look like a neurodegenerative site (as indicated by the authors). The dopaminergic neurons did not look degenerative as you could observe in other manuscripts (PLoS Genet 2010 Aug 26;6(8): e1001084 doi: 10.1371/journal.pgen.1001084; Neurotoxicology 2020 Mar;77:105-113 doi: 10.1016/j.neuro.2020.01.003. Epub 2020 Jan 11). Did you consider to use a different strain (dat-1::mCherry) in order to improve or confirm your data? In my opinion, there are several points in the figures that could be indicated as an asterisk. Please clarify this point.

We realized that the images in the manuscript were not up to a high enough quality, which may have made it difficult in viewing the details of neurodegeneration. In the revised manuscript, we have re-exported these images, so the quality is significantly higher. Figure 6C highlights the areas of neurodegeneration. We are confident that the new set of images will address the concern of the reviewer. Please note that the introduction section (paragraph 5, line 7) has been updated to describe the use of the reporter strain. Specifically, several references for dat-1::yfp strain are included (Salam et al., 2013, Maulik et al., 2017,). The paper by Salam et al. (2013) from our lab has reported the use of dat-1::yfp to investigate DA degeneration in various toxin treated animals. Thus dat-1::yfp is a faithful reporter to study DA neurodegeneration.

Salam, S., Ansari, A., Amon, S., Rezai, P., Selvaganapathy, P. R., Mishra, R. K., & Gupta, B. P. (2013). A microfluidic phenotype analysis system reveals function of sensory and dopaminergic neuron signaling in C. elegans electrotactic swimming behavior. Worm, 2(2), e24558. https://doi.org/10.4161/worm.24558

Maulik, M., Mitra, S., Bult-Ito, A., Taylor, B. E., & Vayndorf, E. M. (2017). Behavioral Phenotyping and Pathological Indicators of Parkinson's Disease in C. elegans Models. Frontiers in genetics, 8, 77. https://doi.org/10.3389/fgene.2017.00077

4) The figure 7 (A-D) present could be changed for better pictures. Some of them as hard to have a clear image from dopaminergic neurons.

We now have provided higher quality and clearer images. Earlier, during the conversion process images did not export at a high quality. Additionally, we have retaken some images and updated the manuscript with higher quality images.

5) I strongly suggest not use the manuscript in preparation as a reference once you have published data.

We acknowledge the reviewer's concerns. Please note that the referenced manuscript has now been accepted in Nature Scientific Reports. Therefore we have updated the citation (also see below).

Taylor, S. K. B., Minhas, H. M., Tong, J., Selvaganapathy, P. R., Mishra, R. K., & Gupta., B. P. (2021). C. elegans electrotaxis behavior is modulated by heat shock response and unfolded protein response signalling pathways. Nature Sci Rep. (in press). https://doi.org/10.1038/s41598-021-82466-z

Comments from Reviewer 2

The manuscript investigated therapeutic effects of TP5 in PD models. The truncated peptides supposed to block Cdk5/p25 complex and authors demonstrated the important role of TP5 to inhibit Cdk5/p25 activity in PD pathogenesis. However, the major concern is that it will be appreciated if representative images/data could be presented with statistical quantification in the figures 3/4/5/8. Some other concerns as the following:

As requested by the reviewer, we have added the statistical analysis in each of the figures' captions.

1. It seems like that the synthetic peptide could penetrate plasma membrane. Does it contain any cell-penetrating peptide sequence?

We have added the necessary information to the introduction section, specifically paragraph two, line two. Specifically, this synthetic peptide contains an 11 amino acid sequence derived from the transactivator of transcription (TAT) protein that is conjugated at the C terminus. The TAT protein not only penetrates plasma membranes but facilitates the passage of the blood brain barrier as well.

2. In figure 4, the data showed the similar toxic effects of TP5 and SCRM TP in SH cells which complicated the interpretation of the data.

The previous graph may not have been very clear, therefore we have replotted it to more clearly show the difference between the results. The significance of numbers has been added in Figure 4 legend along with p values for clarity.

3. In section 3.4, the title was not specific to address at which levels of Cdk5/p25 was increased. The authors immunoprecipitated Cdk5 with specific antibodies for kinase activity assay, did TP5 also block the interaction of Cdk5 with the antibody? It would be great to show the quantity of immunoprecipitated protein by western blot.

We have revised the following section title accordingly. The new section title is “TP5 blocks Cdk5/p25 activity in C. elegans following exposure to PQ”

Please note that the quantity of immunoprecipitated protein by Western blot has been previously reported in two papers published by the lab of one of the authors of this manuscript (Binukumar et al. (2014) & Binukumar et al. (2015).

Binukumar, B. K., Shukla, V., Amin, N. D., Grant, P., Bhaskar, M., Skuntz, S., Pant, H. C. (2015). Peptide TFP5/TP5 derived from Cdk5 activator P35 provides neuroprotection in the MPTP model of Parkinson’s disease. Mol Biol Cell, 26(24), 4478–4491. https://doi.org/10.1091/mbc.E15-06-0415

Binukumar, B. K., Zheng, Y. L., Shukla, V., Amin, N. D., Grant, P., & Pant, H. C. (2014). TFP5, a peptide derived from p35, a Cdk5 neuronal activator, rescues cortical neurons from glucose toxicity. J Alzheimers Dis, 39(4), 899–909. https://doi.org/10.3233/JAD-131784

Specifically, these papers have described in vivo experiments performing the kinase assay with the same trends as our results in Figure 8. The Western blot confirmed the quantity of Cdk5 antibody, p25 and p35 antibody. Using tubulin as the loading control, Cdk5 expression was elevated in MPTP treatment, and these levels did not change with TP5 administered. More importantly, p25 expression following MPTP exposure was downregulated when TP5 treatment was applied, consistent with the results seen in the kinase assay. The results indicate that TP5 selectivity blocks CDK5/p25 activity, and CDK5/p35 activity. Similar experiments have also been done following glucose treatment due to its risk towards Alzheimer’s disease. We have included this information in the discussion section, paragraph 8, line 7 of our manuscript

Accept Letter

Dear Dr. Mishra,

Thank you for submitting your manuscript to Current Research in Neurobiology.

I am pleased to inform you that your manuscript has been accepted for publication.

Please submit a high resolution version of the graphical abstract at the paper proofing stage.

Also please let us know if you would be interested in an Author Q&A to help publicise your article or to tell the story behind the science that made it all possible.

Your accepted manuscript will now be transferred to our production department. We will create a proof which you will be asked to check, and you will also be asked to complete a number of online forms required for publication. If we need additional information from you during the production process, we will contact you directly.

We appreciate you submitting your manuscript to Current Research in Neurobiology and hope you will consider us again for future submissions.

Kind regards,

Christopher I. Petkov
Editor in Chief
Current Research in Neurobiology

-------- End of Review Comments --------