Author’s response to reviews

Title: THE EFFECTS OF THIAMINE PYROPHOSPHATE ON ETHANOL INDUCED OPTIC NERVE DAMAGE

Authors:
turgay uçak (turgayucak10@gmail.com)
Yucel Karakurt (dryucelkarakurt@gmail.com)
Gamze Tasli (nurdangamzemumcu@hotmail.com)
Ferda Keskin Cimen (ferdakeskinn@hotmail.com)
Erel Icel (dr_erel@hotmail.com)
Nezahat Kurt (nazahatkurt@hotmail.com)
Ibrahim Ahiskali (ibrahimiznulov@mynet.com)
Halis Süleyman (halis.suleyman@gmail.com)

Version: 3 Date: 30 May 2019

Author’s response to reviews:

Response Letter

Dear editor and the reviewers,

Thank you very much for your encouraging comments. We studied on the points you mentioned and tried to correct all points. We will be very happy if you would re-evaluate the manuscript. Kind regards.

Response to the Reviewer(s)' Comments:

Reviewer 1:
Lucien Bettendorff (Reviewer 1): This work shows protective effects of thiamine pyrophosphate (by intraperitoneal administration) on ethanol-induced optic nerve damage. It seems that thiamine pyrophosphate acts through antioxidant and anti-inflammatory effects. The results are interesting, but purely descriptive. The authors do not discuss by what mechanism thiamine pyrophosphate could have such effects and they do not make any attempt to elucidate this point.
The choice of thiamine pyrophosphate is intriguing. They rely on a previous study showing that thiamine pyrophosphate has better protective effects than thiamine in alcohol-induced hepatic damage (reference 10). How could this be explained as thiamine pyrophosphate is a heavily charged molecule that does not diffuse through membranes and there are no known transporters for this compound in plasma membranes (in contrast to thiamine). It is possible that thiamine is rapidly eliminated by the kidneys (any excess of the vitamin is eliminated), while thiamine pyrophosphate, which must be dephosphorylated prior to transport, acts as a thiamine buffer. Administration of thiamine pyrophosphate might lead to a slower, but longer lasting thiamine accumulation in the blood than thiamine administration. With the latter, blood thiamine concentrations would rapidly peak with the excess being eliminated. It would be very interesting to check this hypothesis by measuring thiamine concentrations in the blood.

In fact we tried to discuss the mechanism of thiamine thyrophosphate administration by preventing oxidative stress and inflammation. However, we did not work at molecular level which may be the topic of another study. We also did not measure the serum thiamine concentrations at different time periods, which is another limitation of this study. We also did not measure the serum or tissue thiamine concentrations at different time periods, which is another limitation of this study. Determination of serum or tissue thiamine concentrations would elucidate the effectiveness of administered thiamine pyrophosphate. We added this point to the limitations.

The authors should not use the term "total glutathione (tGSH)" which normally refers to the sum of reduced and oxidized glutathione. As they use DTNB to measure GSH it is evident that they only measured reduced GSH as well as other thiols. Similarly at page 6, line 2 (and elsewhere), they should replace "glutathione" by "reduced glutathione".

The terms "total glutathione (tGSH)" are replaced with reduced GSH. Thank you for the encouraging comments.

Table 2 shows the results of biochemical evaluation on tissue sample. What are these tissues. In the Methods section they state they uses heart, kidney and lung tissues. Which one was used in Table 2?

Table 2 shows the results of biochemical evaluation on optic nerve tissue samples.

Minor points

The authors should correct in the abstract: … and only thiamine pyrophosphate (TPG group)… instead of … and only thiamine (TPG group)…

In the abstract, the term ….and only thiamine pyrophosphate (TPG group) is written instead of … and only thiamine (TPG group).

Acknowledgements section
The text below the heading does not correspond to acknowledgements

The term ‘Acknowledgements’ is deleted.
Reviewer 2:

Nasim Zamani (Reviewer 2): Dear Authors
I appreciate the great work you have done to finalize this manuscript. The idea is interesting and the manuscript is well written. However, I think there are some minor points you should correct grammatically. For example, in page 4 line 11, there are two "the"s after each other or in page 5 line 32, we usually check the effect of something ON something else not FOR it. So, I still think a native speaker is better to review your manuscript before possible publication. Otherwise, I found the study very interesting in nature and would like to congratulate you on that.
Good Luck

Thank you very much for your encouraging comments. We corrected the grammatical errors.

Reviewer 3
GENERAL COMMENTS: Authors perform a novel study to understand the pathogenic mechanisms in ethanol induced optic nerve damage and report that thiamine pyrophosphate can limit the damage. Some specific comments to improve the quality of the paper are given below
ADDITIONAL REQUESTS/SUGGESTIONS:
Abstract: Please mention the route of administration and duration of administration of the chemicals into the rats. Give a brief overview of what histopathology was performed on the optic nerve specimen. Please clarify that the 4th group got only thiamine and not TPP.

In the abstract section, the route of administration and duration of administration of the chemicals are mentioned. The fourth group got thiamine pyrophosphate only, and this point is clarified.
Introduction: Generally well written. Authors can mention that ethambutol induced oxidative stress has been shown histopathologically in the past with TPP being effective in preventing this.

In the Introduction section, ‘ethambutol induced oxidative stress has been shown histopathologically in the past with TPP being effective in preventing this’ is mentioned.
Methods: Well described. Please also mention handling of the laboratory animals as be the ARVO convention guidelines.

In Methods section, handling of the laboratory animals as were the ARVO convention guidelines and it is mentioned.
Results: Though authors show reduced MDA and higher levels and higher GSH levels in the group that received TPP, they do not study how these influences were achieved i.e. a gene based analysis for activity of MDA and GSH genes would have been very useful.
The damaging effects of ethanol via the oxidative pathway has been shown multiple times before, especially in neurologic tissue. Hence the same is expected in the optic nerve. There is nothing novel in this finding.
For future research, authors may do well to introduce TPP at varying time intervals after starting ethanol administration and sacrificing the rats at different (and pre specified) time points to see the time-bound beneficial effects of TPP and reversibility of the optic nerve damage on histopathology.
Your comments regarding the results are highly encouraging and we mentioned those points in conclusion section. Thank you very much.