Author’s response to reviews

Title: Histone deacetylase inhibition by Entinostat for the prevention of electrical and structural remodeling in heart failure

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Author’s response to reviews:

Dear Professor Wainford, dear Editorial team,

Thank you very much for reviewing our manuscript and considering it for publication. On behalf of my co-authors I would like to resubmit the above named manuscript to "BMC Pharmacology & Toxicology". Following the advice of your reviewers, we have carefully revised the manuscript. All changes have been highlighted in the text. We hope that you and your reviewers will now find the manuscript appropriate for publication in your journal. Below please find our point-by-point answers to the reviewers’ comments.
With very kind regards, sincerely yours

Philipp S. Lange, MD

Your manuscript "Histone deacetylase inhibition by Entinostat for the prevention of electrical and structural remodeling in heart failure" (PHAT-D-17-00216) has been assessed by our reviewers. They have raised a number of points which we believe would improve the manuscript and may allow a revised version to be published in BMC Pharmacology and Toxicology. Please pay particular attention to the concerns relating to western blot images and the large differences in the loading controls for B-actin.

Response: Western Blot images have been rescanned to optimize clarity. In some cases, differences in the loading controls were observed, however, in these cases, Western Blots were scanned and carefully quantified in order to ensure proper analysis.

Reviewer reports:

Kawa Dizaye, PhD, MSc, HD (Clin.Pharm), BSc Pharm (Reviewer 1):

Page 1 Line 31
KCNH2 and Cav1.3 expression change to KCNH2 and Cav1.3 gene expressions

Response: The manuscript text was corrected accordingly.

Page 3
Line 50:
5 sham-operated rabbits change to Five sham-operated rabbits

Response: The manuscript text was corrected accordingly.

Line 51
What DMSO means?

Response: The abbreviation DMSO stands for dimethyl sulfoxide. The manuscript text was corrected accordingly.

Line 52:
DMSO = Abbreviation

Response: The abbreviation is now mentioned in the manuscript text accordingly.

Ajay Chowdary Donepudi (Reviewer 2): Manuscript on "Entinostat prevents remodeling in heart failure" by Freundt et al shown interesting data on effects of a Hdac inhibitor on electrical and structural remodeling in heart failure. This manuscript shown some interesting changes in fibrosis and ion channels protein expression with Hdac inhibitor administration. Although this manuscript has some interesting data, authors did not explained data well in manuscript. Data in this manuscript showed entinostat administration itself has some changes in protein expression and % EAD values similar to paced animals. These changes by entinostat were not mentioned and discussed in the manuscript. Here are my major concerns-

1. Authors need to discuss effects of entinostat in non-paced animals.

Response: The discussion was revised accordingly.
2. Western blot images are too blurry. Authors need to provide good images. Why authors only quantified one sample from control non paced animals.

Response: Western Blot images were rescanned in order to improve image quality and to avoid blurry images. The group “DMSO/non-paced” animals encompassed a total of 5 animals that were analyzed and compared to the other groups. The manuscript text has been improved appropriately.

3. Statistics between entinostat treatment and control group are missing. How statistics can be performed with one sample in group. Authors should provide their detailed statistics analysis data.

Response: In the present work, animals of four treatment groups were compared to analyze the pharmacological effects of Entinostat in failing (i.e. rapidly stimulated) hearts: Sham rabbits (without rapid stimulation) treated with vehicle (DMSO) or Entinostat and failing (i.e. rapidly stimulated) rabbits treated with DMSO or Entinostat. Normal distribution was assessed by the Shapiro-Wilk-Test, p > 0.05. Two-sample independent student’s t-tests were used to compare the means of two groups. One-way ANOVA was used to compare means of four samples. Differences with a p value of ≤ 0.05 were considered to be statistically significant. The manuscript text has been improved appropriately.

4. Figure legends in this manuscript are very confusing.

Response: Figure legends have been updated in order to improve clarity and to avoid confusion.

5. One of the significant observation in this manuscript is fibrosis. Images are not clear. Figure legends are confusing for this figure (figure-5). Number of animals mentioned in this figure legend does not match with number of animal mentioned in methods.

Response: The corresponding figure legends have been updated in order to improve clarity and to avoid confusion. In some cases, electrophysiological measurements could not be completed due to perfusion problems that can occur in this experimental model and lead to ischemia.
Therefore, no electrophysiological results are available for these hearts. However, these animals were still used for histological analyses. Taken together, the following number of hearts was analyzed histologically: failing hearts treated with DMSO (n=6), sham-operated hearts treated with DMSO (n=7), failing hearts treated with Entinostat (n=6) and sham-operated hearts treated with Entinostat (n=9). The electrophysiology of 5 sham-operated hearts treated with DMSO and 5 failing hearts treated with DMSO and 8 sham-operated hearts treated with Entinostat and 4 failing hearts treated with Entinostat were recorded at the Langendorff apparatus and analyzed subsequently.

6. Authors need to do thorough revision of manuscript for grammatical errors.

Response: The manuscript was revised thoroughly in order to avoid any grammatical errors.