Author’s response to reviews

Title: Efficacy and Safety of a Novel Naltrexone Treatment for Dry Eye in Type 1 Diabetes

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Author’s response to reviews:

REVISIONS TO MANUSCRIPT BOPH-D_18-00385-R1. Thank you to both reviewers. The required clarifications have made the manuscript stronger. All comments (copied as originally written without editing) have been addressed. Responses are provided in red font. Inasmuch as possible, corrections have been included in the text of the manuscript on same page as indicated by reviewer.

Technical Comments:

1. Missing Title Page. Response: Now included

Editor Comments:

The paper is well written and carefully organized. The authors needs to address the concerns raised by the reviewers before considering the paper for publication.

BMC Ophthalmology operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.
Reviewer reports:

Alvin Jeffery Munsamy, M.Optom (Reviewer 1): Title: Consider adding "...treatment for dry eye in diabetes."

Full title: Consider revising "...Diabetic Dry eye" to "...Dry Eye in Type 1 Diabetes" Response: Title has been changed as suggested.

Abstract:

pg 2; line 22: specify Schrimers1 or 2? Response: Inserted in Abstract

Background:

p4; line 7: "Dry eye disease(DED)" specify if its correct to say "Tear Defecient Dry Eye (TDDE)" as opposed to "evaporative dry eye(EVE)" Response: Corrected on pg4, lines 7-8.

p4;line 14: "If left untreated DED may cause pain, corneal ulceration and potential loss of vision"- consider adding the word "severe DED" as not all dry eg mild directly translates to this. Response: Corrected as suggested, page 4, ln14

p4; line `9: "5-34%"; I see this was referenced but can you alert this reader where in general ; like "North America" which is what I can grossly infer from the author and publication names?? Response: clarification made – worldwide on page 4, line 9

p4; line 22-24: ".....(in particular diabetes)" consider "...(including type 1 diabetes)" Response: correction made on pg 4, lines 22-24

p4 ; line 34: consider changing "[3.5-9] to [3, 5-9] Response: corrected

p4; line 41: "...primary prescription....clinical dry eye...Restasis...." : I humbly beg to differ that restasis is the primary prescription treatments; "primary" implies the first line line??; secondly "clinical dry eye" in the context of restasis consider "... clinical TDDE dry with inflammatory etiology" which sounds more accurate. Response: removed ‘primary’ and included reviewer’s suggestion

p4; line 51: " ....lengthy period of application..." please specify what is "lengthy" so it may imply when reading the results of Naltrexone the reader will straight away know in comparison. Response: clarification made on pg 4.

p4; line 58: "..through punctal plugs or surgical treatment through occlusion...for chronic dry eye syndrome"...that statement is far reaching and includes dry eye with an inflammatory etiology
where punctal occlusion is contraindicated to increase tear retention as it keeps the inflammatory mediators on the ocular surface longer. If you disagree please support with a citation. Response: statement has been supported by a citation. Page 5.

p5; line 22: "...diabetic animals [16-18]"; these citations only refer to rats so the use of the word animal is again far reaching, maybe add other citations to other animals or mammals? It is concerning as these are self citations and it should be clearer to say mammals/rats. Response: mice and rats were studied, page 5 now reflects both species.

p5; line 49: .."1000-fold" may be seen as a sensational number but its not comparing directly topical concentrations to systemic and in some light an unfair comparison . I stand to be corrected but it appears the author is answering the safety hypothesis of this paper before presenting the results and suggests its very safe already because the concentration is lower! Consider re-phrasing by simply recording the concentration and not prescribe to the reader, in the interest of impartiality. Response: pg 5, last sentence: clarification made that the systemic treatment is 1000-fold higher; reworded statement as we definitely did not know the answer before doing the study.

p6; line 22-26: Did these bridging studies that proved the safety; convey any behavioral changes considering Naltrexone is primarily used to treat alcohol and opioid dependence. Response: statement that no behavioral changes were noted has been added to page 6.

Methodology:

p6; line 39: The use of male Sprague-Dawley rats- was the gender bias premneditated?; if so please substantiate. This questions the background (p4; line 22) where the female gender is reported to have an increased risk. With respect, was the choice of male rats used to ensure a performance bias? Response: yes, gender was premneditated to be consistent and complementary to our preclinical published work. Statement has been added, last line page 6.

p7; line 5 "5 weeks"; line 12 "70\%".. I am sure these were not an arbitrary choice- please provide a brief reason or insert a citation. Response: Citations have been included on page 7 referencing selection of 5 weeks.

p7; line 34: "1mm widex17mm long": This is not a standard Schirmer width. How was this validated. Can you elaborate how this augmentation differs from standard use and if done in one minute what is regarded as normal. What informed this approach and if a pilot was done using this augmented approach. THIS IS VERY IMPORTANT AS IT IS THE MAIN INDICATOR OF THE SUCCESS OF THE
INTERVENTION. Response: Initial tests were performed with the entire strip and were deemed too wide for the rat cul-de-sac. Other publications suggested cutting the strip to 1 mm wide in order to be able to insert into the center of the cul-de-sac.

p8; line 19: was the IOP measured at the beginning of the study? Response: IOP was measured on a subset of non-diabetic rats.

p11; line 49: how was IOP measured? The asymmetry in the treatment group to the untreated group was significantly higher even after 10 days of treatment- if its implied why I apologise for missing it- please explain why? I am concerned especially since on pg13; line 17 where it is mentioned the optic nerve was not adequately examined? I am concerned about the neural impact of the drop over time. Response: The IOPs were measured in both eyes of rats, with only the right eye receiving active drops. Although the optic nerve per se was not examined, the animals had blink reflexes and normal IOPs following naltrexone treatment.

RESULTS:

For Figures 1-3; considering there is more than one test for tear secretion and to contextualise better can the author replace the label of y-axis from "tear secretion" to "Schirmer’s 1" or even "Modified Schirmer 1". Response: Y-axis labels in Figures 1-3 have been changed to Schirmer’s 1 test (mm)

Fig 4: no p-values as show in fig 2? only mention on p11; line 38- are all other observer insignificant? The corneal sensitivity results on pg 11; lines 19-46 is not explored in the discussion as a palliative cure for diabetics living with dry eye? any reason? ALL THE RESULTS SHOW AN IMPROVEMENT BUT ITS NOT CLEAR IF THE DRY IS CURED? THE SCHIRMERS VALUES ARE NOT DEFINED IN THE METHODS FOR VALUES THAT REPRESENT DRY VS NO DRY EYE. Response: Figure 4 has levels of significance included. The comparisons are between baseline T1D levels and post-treatment.

Comments regarding “cured” dry eye have been added. The authors do not intend to suggest that topical NTX cures dry eye - requiring no further treatment. In fact, the extinction studies suggest that the “dryness” returns after a few days of no topical treatment.

The definition of “dry” vs “non-dry” is provided on page 7 as baseline Schirmer values less than 70% of the mean Schirmer scores for normal rats.

Corneal sensitivity comments are added into the Discussion on page 16.
DISCUSSION:

p14; line 7: which pre-clinical studies? Response: published studies have been clarified and cited on page 14, line 7.

p14; line 19: "..longer than previously reported and spanned 10 days" ; what exactly are you comparing it to? how much longer? over how many days in other drops? I am not convinced this drop is better as I am convinced its an alternative using an alternative M.O. and that's perfectly fine and advocating its more efficient judging from the results is far reaching in my humble opinion. Response: Previously published data on topical treatment of dry eye in type 1 diabetic rats were obtained in studies that were only 7 days in length --- hence 10 days is longer. The NTX eye drop does have an alternative M.O. as it likely blocks the OGF-OGFr regulatory axis; this formulation is more than just fluid (similar to OTC eye drops).

p14; line 27: insert citation or say what it is. Response: citation has been added on page 14.

p14; line 34: "...reversed dry eye": The use of reverse implies cured- was it reversed to normal- this goes back to methods and what is regarded as cured with the augmented Schirmer's used. Response: The topical NTX treatment restores tear fluid volume in diabetic rats to that of baseline in naive rats – hence it reverses the dry eye or restores tear fluid levels. However, this restoration of “normal tear volume” is not maintained if NTX treatment is ceased. We have added a statement regarding extinction of the effect on page 15.

p14; line 41: "..normal tear fluid".. I want to challenge the accuracy of this fluid levels? Response: Our definition of normal tear fluid is that of naive male rats at baseline.

p14; line 46-51: from a time perspective for how long? Response: 30 days

p14; line 58: " It is.. previous published..." insert citation. Response: citations included on page 14

p15; line 14: I am not convinced about the IOP unless it was high to start of with which was not shown anywhere. Response: published IOPs for male Sprague-Dawley rats indicate IOPs as high as 25 mm Hg.

p15; line 29: "...most likely" this appear very vague and almost arbitrary; sure the mechanism of action must be more concrete if not state why. Response: Sentence was reworded. We are convinced of the mechanism that we can prove, but it may not be the only pathway.

p15; line 39: "...restore both tear production and corneal sensitivity.": Is it tear retention or tear production? as previous work by the author evidenced in the reference 13-21 - is the restoration of wound healing allow for the tearfilm to attach better to the cornea and thus improve sensitivity and is this co-dependant on tear retention or tear production? I say this because there NO mention of the lacrimal gland ( responsible for tear production) anywhere. Can I request the
authors clarify this concern? Or is the premise that the use of Schirmer's is enough to infer tear production and improvement in sensitivity disregarding the previous work of corneal wound healing? Is the improvement in Schirmer's an observation noted in previous studies and this is an "off label" exploration for main stream consumption of dry eye vs niche treatment of corneal wound healing? All this is questionable in the absence of a direct pharmacological mechanism of action on the eye. It is worth the authors attention. Response: We did not test tear retention in this study as the focus was the tear volume following the novel topical treatment. This manuscript is not intended to delineate mechanistic pathways.

REFERENCES:

p17; line 49: date accessed? Response: Date accessed has been included.

Sarah Atkinson (Reviewer 2): This is very interesting paper, highlighting the use of naltrexone with an alternative delivery system than that previously used. There are some minor comments which may need to be addressed before publication, these are as follows:

1. The prevalence of Dry Eye Disease (DED) is higher in females than in males, does this have ramifications for the sex of the animal used within the study? Response: We acknowledge that females have greater prevalence rates for DED. Our PoC studies will include male and female subjects. Funding for this project limited our ability to include both genders.

2. Within the Background, Lifitegrast is mentioned as a primary prescription treatment for DED, it might be worth including the efficacy of this treatment (if possible) to provide more evidence for the need for a new drug.

Response:

As of Oct 2018, I was unable to find a published rate of efficacy. Website indicates “some patients” and reduced signs of dry eye after 12 weeks.

https://www.xiidra.com/safety-and-studies

3. A sentence needs to be included on why it was necessary to replace the antibiotic-containing carrier Vigamox, within the Background section. Response: Explanation for the transition from Vigamox to a proprietary eye drop has been added on page 5 (last paragraph). It was done because Vigamox contains an antibiotic and should be avoided for extended periods in humans. In addition, the new formulation is a proprietary substance.
4. Within the methodology section, the age of the animals used has not been included, this would be helpful to confirm that the age between different experimental animals is consistent. Response: Additional information on the age at the onset and conclusion of the studies has been added on page 7.

5. Within the treatment section of the methodology, it would be helpful to provide a comparison of doses between NTX-001 and NTX dissolved in Vigamox, eg. NTX-001 is in ug/ml and NTX dissolved in Vigamox is in M. This could be provided elsewhere in the manuscript if more appropriate. Response: Dosage conversions are now included on page 14 of the Discussion.

6. On page 7 of the manuscript, within the methodology, it states 'Following cessation of treatment, tear volumes were measured daily in a subset go rats to assess when the dry eye returned', it would be useful to have this subset defined, for example numbers and which experimental groups? Response: Clarifications have been included on page 7. In general, extinction was studied in 5 rats receiving NTX-001 treatment.

7. Was Intraocular pressure only measured at the termination of the study? It may have been useful to measure it earlier to define a baseline. Response: IOPs were measured at the start of the experiment and have been included in the text on page 11.

8. Is 3 a sufficient sample size for the group of T1D rats which received buffer? Response: An N of 3 is small, but because we had within animal controls (eye not treated), as well as between group controls, it was not considered a concern.

9. Figure 3 and 4 need to be re-formatted as the labels are unclear. Response: Y-axis labels are redone for Reviewer #1. Graphs have been enlarged, and transported between Excel and GraphPad Prism for clearer images.

10. Within the figures, considering using alternative patterns or legends within the graphs that make it easier to differentiate between groups. Response: See #9 above.

11. Within the results section, it is not clear where the 'Normal' measurements have come from, particularly as the 'Normal' measurements are so much higher than those of the untreated T1D eye. Response: “normal” refers to non-diabetic animals.

12. How is 'appeared to have normal vision defined' for the rabbit experiments? It may be useful to include this within the manuscript. Response: Veterinarians indicated that the rabbits were able to obtain food and water, and responded to technician appearance and handling. This was added to the text.
13. Would it be useful to include some images from the H&E staining?

Particularly where features described in the text are demonstrated. Response: Because there was a lack of pathology, the expert veterinary ophthalmic pathologist did not capture