Author's response to reviews

Title: Inhibition of matrix metalloproteinases attenuates brain damage in experimental meningococcal meningitis

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Author's response to reviews: see over
We are resubmitting a revised version of the manuscript no. 1442691674145168 entitled ‘Inhibition of matrix metalloproteinases attenuates brain damage in experimental meningococcal meningitis’ by Ricci et al. We are grateful for the constructive criticism provided by the reviewers. Following their suggestions, we have added a new figure on survival of treated vs. control mice (Fig. 1), improved the figure on correlation between MMP-9 levels and brain damage by adding the panels relative to control mice (new Fig. 4), inserted a new supplementary table with the statistical analyses on all correlation data (Table S1, Additional file 2), and adjusted the body of the text accordingly.

We believe that the revised version has improved in quality and we hope to have addressed all the concerns raised by the reviewers. Please find below our answers to each specific point.

Reviewer: Christian Ostergaard

The study by Ricci et al describes the role of matrix metalloproteinases in the pathophysiology of meningococcal meningitis, and demonstrates that pretreatment with the MMP-inhibitor, batimastat improves the outcome of the disease. The study documents that the authors successfully have developed an animal model of meningococcal meningitis using live meningococci, which is an important achievement in future research for more effective treatments of meningococcal meningitis.

Major compulsory revisions.

1. A limitation of this study is lack of statistical power, which has to be addressed in the discussion. Pretreatment with MMP-inhibitor have likely not only a beneficial effect on cerebral
bleeding and BBB-integrity, but also on survival (P-value: 0.064, type II-error?). The Kaplan-Meier survival curves should be added as a figure to demonstrate the clinical course of the disease (+/- BB-94 treatment).

We agree with the reviewer’s comment on statistical power. It is plausible to expect a beneficial effect of batimastat not only on brain damage, but also on survival. We may have failed to detect a difference in survival between treated and control animals due to the relatively small number of mice in the groups. In fact, by diminishing the threshold of significance from 95% to 90% (setting an alpha value of 0.1), differences become significant suggesting that larger animal groups could have improved the data. However, a total of 106 mice (inclusive of both groups) would have been necessary to achieve statistical significance (alpha=0.05; power=0.8) according to sample size calculation based on present data. As animal survival was not the primary goal of the study, we decided not to perform additional experiments in accordance with the 3R principle for animal experimentation (Replacement, Reduction and Refinement). In the revised manuscript, we have modified a sentence (page 10, line 7), added a new figure (Fig. 1) containing a Kaplan-Meier survival graph, and made a comment on statistical power in the discussion (page 14, lines 12-14).

2. **Another limitation that should be addressed in the discussion is, what impact it may have on the other results presented that survival was higher in BB-94 group than in the control group (only 20/21 vs. 13/20 mice, respectively, were included in all analyses). Could it be the most severe cases that were excluded from the analyses?**

   We would like to thank the reviewer for his observation. Differences in MMP-9 levels, cerebral bleeding and BBB disruption between treated and control mice might have been larger if deceased control animals would have been included in the analyses. A sentence has been added in the revised version (page 14, lines 15-18).

3. **The correlation analysis in Figure 3 is of little value. The positive correlations between MMP-9 and cerebral haemorrhage/brain albumin are most likely due to the documented differences in**
these data between the two treatment groups. Data of correlation analysis should be shown for each treatment groups individually.

We agree with the reviewer’s remark. In the revised manuscript, we have modified figure 3 (now figure 4), inserted a new paragraph in the ‘Results’ section (page 11, lines 15-22), and made a brief comment in the ‘Discussion’ (page 14, lines 23-25). The new version of figure 4 contains 3 additional panels related to control animals (Fig. 4A-C) and 3 original panels (Fig. 4D-E) that comprise pooled data from control and treated subjects. Pearson analysis on data from control samples showed a significant ($p=0.031$) and a borderline significant ($p=0.054$) correlation for the variables MMP-9/BBB breakdown and MMP-9/bleeding areas, respectively. We have not included graphical results referring to treated mice, as correlations were not significant probably because additional factors, besides MMP-9, may be responsible for the residual brain damage in treated animals. However, we have added a supplementary table (Table S1, Additional file 2) that summarises the statistical analyses for all groups, both individually and separately.

Minor essential revisions.

1. Page 5, line 24. Please, describe briefly the content and values of the coma scale to help reading the manuscript.

To clarify this point, we have included a brief description of the coma scale (page 5, line 25 and page 6, lines 1-2).

2. Page 4, line 17, and page 14, line 11. Please remove “adjunctive”, since BB-94 treatment was not given as adjunctive therapy. It should be stated that future studies should explore, whether anti-MMP treatment would have a beneficial effect on meningococcal meningitis as adjunctive therapy (together with antibiotics).

Following the reviewer’s suggestion, we have removed the term ‘adjunctive’ when inappropriately used throughout the text. In addition, we have included a sentence in the ‘Conclusions’ as suggested (page 15, lines 15-17).
Minor revision not for publication.

1. Page 8, line 15. Please clarify, whether the strong correlation was solely for MMP-9 or for other analysis included in the Milliplex assay.
   The correlation referred to MMP-9 only. The sentence has been clarified in the revised manuscript (page 8, lines 17-19).

2. Page 9, line 15. Rho > 0? What about negative correlations?
   The reviewer’s comment is correct. We have amended the sentence in the new version of the manuscript (page 9, line 18).

3. Page 10. line 7-10. Please remove the less important cytokine data to the end of the result section.
   We understand the reviewer’s concern. However, as cytokine data are limited and do not merit a section on their own, we chose to leave the findings in the introductory chapter of the ‘Results’ section.

Reviewer: A. M. Marceline van Furth
No revisions required.

We look forward to hearing about your decision.
Sincerely yours,

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