Author's response to reviews

Title: Daptomycin in experimental murine pneumococcal meningitis

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Author's response to reviews:

Dear BioMed Central Editorial Team,

Thank-you for your thoughtful review of our manuscript (MS: 2617297792487681) entitled: "Daptomycin in experimental murine pneumococcal meningitis." Please find below our responses to the referee comments.

Referee 2:
- Minor Essential Revisions

1. Was the mortality rate of the model 0%? Did all infected mice survive until 24 h after infection?
   - There was no mortality. This has been clarified in the Methods section.

2. Line 115: The whole brain sections where scored for inflammation, neuronal necrosis, and apoptosis…
   - The whole brain sections were scored for inflammation, neuronal necrosis, and parenchymal apoptosis; this has been clarified in the revised manuscript.

3. Line 165: "The amounts of inflammation and apoptosis of brain parenchymal cells where equivalent…
   - The presence of bacteria in the histopathology specimens was descriptively recorded.

- This finding is rather unexpected and is somewhat contradicted by the data presented on line 170-172 "the density of bacterial cocci…appeared to be lower…". Here the presentation of the data on "inflammation and apoptosis of brain parenchymal cells" and a statement on the methods used for quantification of bacterial density would be helpful for interpretation of the findings discussed on lines 229-233.

   - The presence of bacteria in the histopathology specimens was descriptively recorded.
4. Table 1 and Figure 3: Data at 0 hours is lacking in the table on groups dexamethasone/ plus antibiotics while data is presented in Figure 3. Has the data at 0 h been pooled from all treatment groups? Please clarify.

- The 0 h data was pooled from all treatment groups. This is clarified in the revised Table 1.

- Discretionary Revisions

Abstract and elsewhere: "intrathecally" could be replaced by "Into the cisterna magna" or "intracisternally"
- Done

- Lines 87-88 and 92-93: Duplicate statement of : "96 animals where divided into…"
- Corrected. Thank-you for bringing this to our attention.

Referee 3:

All methods were relevant and well executed, though CSF WBC concentrations were compromised by blood contamination due to traumatic CSF tapping (how often? Could be adjusted, if a corresponding blood samples were obtained) and that technical problems resulted in bioluminescence imaging of only 73% of animals. In addition, this method was of limited value in the present study; despite bioluminescence imaging is a highly relevant method for studying the pathogenesis and pathophysiology of bacterial meningitis and therefore might be of relevance in future experimental studies from this group.

- We have added a comment regarding the value of in vivo biophotonic imaging to the revised manuscript. Unfortunately, we were unable to calibrate the spinal fluid leukocyte versus peripheral counts.

Pharmacokinetic analysis would have been more complete by studying CSF antibiotic concentration and not only serum concentration, because adjunctive therapy with dexamethasone potentially could reduce CSF antibiotic penetration.

- Unfortunately, we were unable to measure either daptomycin or vancomycin by bioassay in undiluted CSF from uninfected mice not treated with dexamethasone.

Treatment with daptomycin (without concomitantly dexamethasone therapy) was previously shown to cause less brain damage than conventional antibiotic therapy. Moreover, treatment with dexamethasone, when combined with ceftriaxone, caused increased hippocampal injury than treatment with ceftriaxone alone. Therefore, the present study would have been more interesting, if experimental groups not given dexamethasone also were included.

- We agree that it would have been interesting if experimental groups not given dexamethasone had been included. Unfortunately, due to resource limitations, we were unable to do this.

Minor Essential Revisions.
Background, 2. paragraph. Please add the results of previous experimental meningitis studies with daptomycin to clarify to relevance of the present study.
- This topic is addressed in the discussion

Methods, line 64. “Serotype 3 is one of the most common pneumococcal serotypes” will be more correct.
- Changed, as suggested.

Methods, line 89. 3 x 10e4 CFU of bacteria.
- Changed, as suggested.

Methods, line 89, please give doses of ketamine and xylazine.
- Done

Methods, line 98. Were mice reanesthezed with ketamine and xylazine?
- This is correct and has been corrected in the revised manuscript.

Methods, line 100-2. Since lower detection limit was 20 CFU/mL, please indicate that 50 µL of undiluted CSF also were plated.
- The lower limit of detection was 20 cfu/ml because 50 µl of CSF was incubated for culture. 25 µl of CSF were used to make dilutions and 25 µl of CSF was spread on the surface of a sheep blood agar plate. The plates were incubated and the diluted CSF not used for subsequent dilutions or culture was also incubated.

Figure 1. Labeling seems not to be correct. DX = control? Control = DP?
- Corrected; we regret the error.

Figure 3. Confidence intervals are overlapping and therefore difficult to evaluate. Please separate, if possible.
- Done

We hope that you find the manuscript substantially improved.

Sincerely yours,

Robin Patel, M.D.