Author's response to reviews

Title: Antifungal wound penetration of amphotericin and voriconazole in combat-related injuries: case report

Authors:

Kevin S Akers (kevin.s.akers.mil@mail.mil)
Matthew P Rowan (matthew.p.rowan.vol@mail.mil)
Krista L Niece (krista.l.niece.ctr@mail.mil)
John C Graybill (john.c.graybill.mil@mail.mil)
Katrin Mende (katrin.mende.ctr@mail.mil)
Kevin K Chung (kevin.k.chung.mil@mail.mil)
Clinton K Murray (clinton.k.murray.mil@mail.mil)

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Author's response to reviews: see over
Re: Antifungal wound penetration of amphotericin and voriconazole in combat-related injuries: case report
Kevin S Akers, Matthew P Rowan, Krista L Niece, John C Graybill, Katrin Mende, Kevin K Chung, Clinton K Murray

Dear BMC-Infectious Diseases Editorial Staff:

We would like to thank the reviewers for their time and careful consideration of our manuscript entitled “Antifungal wound penetration of amphotericin and voriconazole in combat-related injuries: case report” for publication in BMC-Infectious Diseases. We have revised the manuscript and believe the reviewers’ concerns have been adequately addressed, which has helped strengthen the paper significantly. A summary of the reviewers’ concerns and the changes made is given below.

Reviewer 1 (Lass-Floerl):

“Why did the authors use natamycin as a standard to test L-amphotericin B? Why did the authors set up an itraconazole standard to measure voriconazole?”

The natamycin and itraconazole serve as internal standards to correct for the loss of analyte during sample preparation and analysis. Natamycin was used as an internal standard for HPLC analysis of amphotericin B in the previously described method (Lambros et al., 1996) used in our study. The published method (Gordien et al., 2009) used for the analysis of voriconazole in our study evaluated the accuracy, precision, and recovery percent for five azoles simultaneously. We chose itraconazole as our internal standard due to the separation in retention times and similar recovery percent as voriconazole, although any of the other azoles used in this study would have functioned well as an internal standard in our assay. We have updated the description of the methods in the Case Presentation section (lines 112-143) for clarity.

“Did the authors test for amphotericin B or indeed test L-amphotericin B?”

This is a very good point and we thank the reviewer for bringing it to our attention. Patients were treated with liposomal amphotericin B (L-AmB) due to improved tolerability over amphotericin B, but the HPLC analysis we used evaluates the concentration of total amphotericin B, not just the portion of amphotericin remaining in liposomal form. We have updated the wording throughout the manuscript (multiple locations) to highlight that patients were treated with L-AmB, and samples were analyzed for the concentration of amphotericin B.

“Why did the authors collect tissue samples of patient 1? Did the authors investigate tissue samples or surgical debridements?”

Tissue samples were collected as part of clinical care, rather than research, and by members of the clinical care team in Germany rather than by the authors in the United States. Prior experience with cases of soft-tissue invasive fungal infection in combat injuries led to creation of a clinical “blast protocol” which included tissue sampling in high-risk patients in order to achieve earlier identification of invasive fungal infection. This clinical protocol is detailed in Reference 8. We have added the words “as part of clinical care” (line 163), and “performed at the discretion of the clinical care team” (Line 166) to avoid confusion as to why the samples were collected.
“What was the reason classifying this patient as suffering from a fungal infection?”

With histopathologic evidence of angioinvasion, Patient 1 met the case definition for proven invasive fungal infection (IFI). This definition was applied, as cited, from Reference 9 (Weintrob et. al, Epidemiol. Infect., 2014), which reported the epidemiology of combat blast-associated IFI: “A proven IFI case was confirmed by angioinvasive fungal elements on histopathology, whereas a probable IFI case had fungal elements identified on histopathology without angioinvasion…. A possible IFI described all cases in which wound tissue grew mould; however, histopathology was negative for fungal elements.”

“I suggest to shorten the discussion.”

We have removed the paragraph speculating on possible alternative treatments which may have improved wound penetration (4th paragraph of the discussion in the previous version).

Reviewer 2 (Afeltra):

“It would be useful to add in the text patient 2a and 2b as in mentioned in Table 1 and Figure 1 in order to be more clear.”

We have updated the wording in the text (lines 192 and 202) to increase congruence between the text and the figure/table.

“It would be interesting to clarify what kind of hyphae were observed in the direct microscopic examination.”

For tissue specimens that failed to grow a mold in culture, the histopathologic description of the hyphae was non-septate and branching. This description has been added to line 164.

Sincerely,
Kevin S. Akers, MD
United States Army Institute of Surgical Research
JBSA Ft. Sam Houston, TX
Kevin.s.akers.mil@mail.mil