Author's response to reviews

Title: Experimental study on cryotherapy for fungal corneal ulcer

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Author's response to reviews: see over
Dear Editorial Board,

On behalf of all my colleagues, I would like to submit a manuscript entitled “Experimental study on cryotherapy for fungal corneal ulcer” to your prestigious journal, BMC Ophthalmology, as a “Research Article”. The content has been submitted to BMC Ophthalmology and decided as rejection and resubmission. The paper ID was 1300632591138507. In the version, we have reorganized the manuscript according to the comments of reviewers and editors. And the language has been thoroughly edited by Edanz English Editing, China.

Fungal keratitis is a leading cause of visual impairment worldwide. Filamentous fungi, especially Aspergillus species, Fusarium species, and the yeast Candida species are the predominant cause of fungal ulcers in tropical regions and are thought to be particularly virulent. Once in the corneal stroma, the conidia germinate, hyphae spread through the tissue, can penetrate into the anterior chamber which cause severe corneal opacification, visual impairment, and blindness. Currently, fungal keratitis treatment is largely dependent on anti-fungal agents. In our study, using rabbit models of cornea infection, we firstly combine cryothermal treatment with antifungal agents. The continuous SEM and TEM observations indicate the cryothermal treatments largely inhibit the fungal mycelium growth by destructing fungal cell structures. The typical cryotherapy was significantly effective for fungal corneal ulcer curing. Meanwhile, different fungi show different susceptibility to the combined treatment: Edanz English Editing, China. Our result established a new combined method (cryotherapy and antifungal treatment) and achieved effective curing results, which
will help facilitate the practice of fungal keratitis curing.

All named authors have agreed to the publication of the work, and the manuscript does not infringe any other people’s copyright or property rights. The work in whole or in part has not been submitted elsewhere for publication. If you have any question please feel free to contact the corresponding author Dr. Minghong Gao, Department of ophthalmology, Shenyang Military Area Command, Shenyang 110016 China

We hope you will make a favorable decision on our manuscript, and we look forward to hearing from you.

Yours sincerely,

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Response to reviewer 1

The language has been thoroughly edited by Edanz English Editing, China and we have carefully revised the manuscript to avoid any grammar and spelling errors.

Q1 Line77; “Candida, Fusarium and Aspergillus” Authors should mention information of organism in detail such as species and an origin. Although authors described “standard strains” in line 84, they should mention strain name or ATCC number. Moreover susceptibility of fluconazole and natamycin should be checked.

A1: The specific name and origin of the fungal species were added in the manuscript. The statement “standard strains” in previous version was incorrect. The isolates used in the present study were provided by Department of Dermatology, Chinese Medical University and hasn’t been preserved in any microbiological culture collection center.

Q2 “1×10^7 cfu/ml” How was organism number measured? Did they use optical density of culture media or microscopy to counts spore?

A2: The number was determined using microscope at 10× magnification.

Q3 To establish fungal keratitis in rabbit, previous works used intrastromal injection of fungi after administration of immune suppressive agents or steroids. Authors establish new methods using keratoplasty. Authors should mention the reason for using new methods.

A3: We used the internationally accepted corneal injection method combined with corneal scratch method to improve the success rate of modeling and the reason has been added in the manuscript.
Q4 Authors should describe scoring in detail.

A4: The criteria of scoring were listed in Table S1.

Q5 Authors should describe rabbit number per group.

A5: The detail information of animal grouping was shown in Table S2.

Q6 Authors should describe concentration of fluconazole and natamycin

A6: Concentration of fluconazole was 2 mg/mL and concentration of natamycin was 2.5 mg/mL. The concentration has been added in the manuscript.

Q7 I am wondering when authors performed the cryotherapy.

A7: The cryotherapy was conducted three days after the model establishment. The information has been added in the manuscript.

Q8 “corneal lesions were removed” Does it mean that corneal graft were removed? If so, how was the graft of control group (pure medication therapy)?

A8: The graft was removed 48 h after the model establishment. The corneal lesions were removed right before the cryotherapy using microscope.

Q9 Although authors described structure of fungal cells in SEM and TEM, I could not understand structure in Figures. Figures does not show scale, and authors should use arrow or arrowhead to indicate structural changes. Authors should take care about SEM or TEM image. To show objectively results, we should show low and high magnification of images and indicate how rate of cells show changes. Estimation of structural change is subjective. Authors should describe how many eyes were observed for changes.

A9: The figures have been modified and scales have been added in the figure legend.
Arrows that indicate the detail changes in morphology have been added. Detail information of treatment efficiency was shown in Table 1 and Table 2.

Q10 Findings of slit lamp is also subjective. Authors described scoring in method section, however scoring does not show in results section. How many eyes did show findings they described in each group? Figures of PAS staining and HE staining look very unclear, and figure should indicate scale.

A10: The detail information of treatment efficiency was shown in Table 1 and Table 2. The figures has been modified and figure scales have been added in figure legend.

Response to reviewer 2

The manuscript has been edited according to your suggestion.
Response to editor

The language has been thoroughly edited by Edanz English Editing, China and we have carefully revised the manuscript to avoid any grammar or spelling error.

Q1: Under Animal Model Establishment, it is stated that previous models use intrastromal injection or keratoplasty methods. Please provide references for both methods, and indicate the success rates of these earlier methods, so that your success rate can be compared to previous models.

A1: The reference has been updated and the success rate of intrastromal injection method has been added to the discussion. However, for the keratoplasty method, which was based on a conference report, we haven’t got the response from the authors and have no access to the detail data of the success rate. We expected that the combined method used in the present study could ensure the success rate and the feasibility of the model establishment for different fungal species, but we didn’t compare the success rates between different studies.

Q2: I notice in the Discussion that reference 11 is given, but this should be ref 14. Please re-check all reference numbering in the manuscript, to ensure it is correct.

A2: The numbering has been corrected in the manuscript.

Q3: Table 1 values appear switched and incorrect values are given, which do not agree with the totals from Table 2. Please revise Table 1.

A3: The values in Table 1 have been revised.
Q4: It should be stated if the scoring criteria in Table S1 is new, or alternatively a reference should be given.

A4: The scoring criteria in Table S1 are based on the Ref. 16 and 17. However, we have done some modification to the criteria according to our experiment need.

Q5: Under Surgical Procedure, it is stated that the lesions were removed using microscope. Does this mean a laser dissecting microscope, or was some other manual instrument used? Please specify in detail how the lesions were removed.

A5: The surgical procedure was conducted under a stereo microscope. Centers and edges within 1 mm of ulcer were carefully removed to expose the ulcer base and health cornea tissue with a sterilized scalpel. The information has been added to the manuscript.

Q6: Captions for Figures 6 and 7 should state 'histological', not historical.

A6: The words have been corrected.

Q7: The captions for the figures are too short and general. Please indicate specific features indicated by the various figure panels, and include arrows on the figures indicating the features. This was suggested in the original review by Reviewer 1.

Q7: The figure legends have been revised in the manuscript. And arrows have been
added in the figures of SEM and TEM as suggested by Reviewer 1.