Author’s response to reviews

Title: The effects of phosphanegold(I) thiolates on the biological properties of Acanthamoeba castellanii belonging to the T4 genotype

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Author’s response to reviews:

Dear Prof. Yuan,

Thank you for your letter and a thorough review of our manuscript. Attached please find our revision. There are two word files, one corrected version (Gold_amoeba_ms) and second with changes shown (marked up manuscript). Page numbers and line numbers in our responses refer to the supplementary file. For your information, our responses to the comments are as follows:

Reviewer’s comments

Reviewer # 1

Q1: (1) Please justify the sentence of "that has attracted increasing interest from the scientific community…"

Response: This is corrected in the revised manuscript. Please see page 3, lines 2 – 4.

Q2: (2) How do you culture the A. castellanii, give a detailed introduction.

Response: This is corrected in the revised manuscript. Please see page 4, lines 15 – 22.
Q3: (3) Why 2mg/ml as the protease substrate?

Response: Our previous studies demonstrated this conc. to be optimal for zymographic assays. This is indicated in the revised manuscript. Please see page 7, line 15.

Q4: (4) What is the result of testing the anti-amoebic effects of phosphanegold(I) thiolates?

Response: Anti-amoebic effects were not observed. This is indicated in the revised manuscript. Please see page 8, lines 4-5 and line 14.

Q5: (5) A related paper is not cited, see "Image processing methods to elucidate spatial characteristics of retinal microglia after optic nerve transection"

Response: We are not sure whether it is an appropriate to include this paper. Please indicate where you would like to cite this and we will do so in the next revision.

Reviewer # 2

Q6: In the current study, the authors examined the effects of phosphane (I) gold thiolates on the biological properties of A. castellanii and found that gold-derivatives had no effect on the viability, growth, cellular differentiation or extracellular proteolytic activities of A. castellanii. These findings indicate A. castellanii has resistant effects to the mental (gold) toxicity and provide an attractive model of metal resistance in eukaryotic cells. The results are of publishable values, however, with some minor problems:

Response: Many thanks for your comprehensive feedback. We agree and believe that the findings are interesting and indicate amoebae as a useful model to carry out further studies in mechanisms of resistance of metal compounds.

Q7: In Abstract: The methods and the results in the abstract are not strictly organized.

Response: This is corrected in the revised manuscript. Please see revised abstract.

Q8: In Methods: 1) Sections "Preparation of A. castellanii cysts and excystation assays" and "Encystation assays" should be better incorporated;

Response: This is corrected in the revised manuscript. Please see revised manuscript, page 6 and 7.

Q9: 2) Explain the reason for the concentrations of AAu1-AAu3 used (i.e. 100, 200, and 300 μM);

Response: Micromolar concentrations are considered as a benchmark to test potential drugs, hence these were used as standard concentrations.
Q10: 3) Please include a section of "Statistical analysis" in Methods.

Response: This is included in the revised manuscript. Please see page 8.

Q11: In Results: 1) The panels in Fig 2B should be better numbered as B1, B2……, the same for Fig 4B, or please refer to the journal style;

Response: This is corrected in the revised manuscript.

Q12: 2) The ordinates in Fig 4A are not correct. Please check.

Response: This is corrected in the revised manuscript.

Q13: In Discussion: Give brief discussion on the possible reasons for the discrepancy that phosphanegold(I) thiolates affects some other microbes as previously reported, but not A. castellanii in present observations.

Response: This is corrected in the revised manuscript. Page 12 and 13 of the revised manuscript.

Reviewer # 3

Q14: Amoebiasis is one of the leading causes of death from parasitic diseases worldwide, and there is a need for the development of new antiamoebic agents. Previous studies have reported that phosphine gold complexes exhibited a different spectrum of in vitro activity against several strains bacteria and fungi. In this study, the author used different assays to show that Acanthamoeba Castellanii (T4 Genotype) is resistant to Phosphanegold(I) thiolates and thus could serve as a model to study mechanism of metal resistance.

1 Although gold complexes have been shown to possess anti-bacteria and anti-microbial activities, very few reports have shown they have anti-amoebic activity except for gold complex with metronidazole. The author has studied Phosphanegold(I) thiolates in the cancer context before. Are there common pathways identified that could be important for Acanthamoeba survival or cyst formation, which suggested it could have anti-amoeba activity? In other words, how is this compound expected to have anti-amoeba activity?

Response: Reviewer is right that some previous studies showed that gold compounds affect Entamoeba histolytica (causative agent of amoebiasis). E. histolytica is a true parasite (not considered free-living). It does not possess mitochondria and hence have entirely different respiratory mechanisms compared with Acanthamoeba. In this study, for the first time we tested the effects of gold compounds on Acanthamoeba. Acanthamoeba is free-living amoebae that is shown to respire under both aerobic and anaerobic conditions, which may explain its ability to resist toxic effects of gold compounds. As gold compounds are known to act on various targets and often inhibiting respiration in microbes and cancer cells, it was logical to test their effects against Acanthamoeba. This is highlighted in the Introduction and Discussion sections.
Q15: 2 How about combining Phosphanegold[I] thiolates with current anti-amoeba drugs, such as chlorhexidine or metronidazole? Will Phosphanegold[I] thiolates enhance efficiency of these drugs, given that the author predicted it could have anti-amoeba effects? How about combined treatment with other drugs in the Amoebistatic assays and encystation assays as well?

Response: This is a good point, albeit combination of drugs makes it difficult to determine molecular mechanisms of action. Metronidazole is not used for Acanthamoeba (but for Entamoeba), but future studies will test combination of gold compounds with current recommended drugs to determine whether their activity is enhanced. This is highlighted in the Discussion section of the revised manuscript. Page 13, lines 7 – 10.

Q16: 3 Some of the Phosphanegold[I] thiolates trended towards a positive results in one or two of the assays, such as the AAu2 in Fig. 2A. Is it possible to have a more significant effect at a higher dose?

Response: Thanks for highlighting this. It is possible and it is now highlighted in the revised manuscript, Page 13, line 7 – 10. We can only say any effects if there is any significant difference. Based on P value, there is no difference, hence we have to indicate that these concentrations give similar effects. Also, any drugs with more than 300uM conc. will prove of limited therapeutic value for clinical applications.

I hope that our revisions are sufficient to allow the publication of our manuscript in the Journal of Negative Results in Biomedicine. We are grateful for the comments as well as the thorough review, which has improved the quality of this work.

Thank you for your consideration,

Sincerely yours

Prof. Naveed Khan

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