Reviewer's report

Title: Therapeutic and immunomodulatory activities of short-course treatment of murine visceral leishmaniasis with KALSOMETM10, a new liposomal amphotericin B

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Reviewer: Abebe Genetu Bayih

Reviewer's report:

Comments for the manuscript entitled “Therapeutic and immunomodulatory activities of short-course treatment of murine visceral leishmaniasis with KALSOME™ 10, a new liposomal amphotericin B”.

The study has been done to test the efficacy and toxicity of a low dose KALSOME™ 10 for the treatment of visceral leishmaniasis in mice. In addition, the study has investigated the immunomodulatory function of the new drug formulation.

It appears that the study is a continuation of another study done on the same drug formulation (Mishra et al, 2013). Basically, the present study has demonstrated that the drug given ONLY TWICE to BALB/c mice at a dose 7.5mg/kg is sufficient to clear Leishmania donovani infection with no hepatic and renal toxicity. Moreover, it has shown that treatment with the drug shifts the antileishmanial immune response to a predominantly Th-1 type. Thus, the study has shown that KALSOME™ 10 is a promising drug formulation for short-term treatment of kala-azar.

Generally, the experimental data are well presented in the manuscript in a very concise but informative manner. In addition, the manuscript is well-written and the message is easily understandable. However, the design of the experiments missed few fundamental components such as the absence of proper controls. I believe, the authors should address the following issues in order for the manuscript be accepted for publication on BMC Infectious Diseases.

A) Major Compulsory Revisions:

1. The experiments on the efficacy and immunomodulatory of the drug did not involve controls such as the drugs that are currently used for treatment of VL patients such as AmB and AmBisome. Without controls, it is difficult to determine where the efficacy of the new drug falls as compared to the known drugs following the experimental procedures used in this study. Please clarify why a “positive control" was not included in the study.

2. As described in the introductory part of the manuscript, the authors appear to rationalize the usefulness of a liposomal amphotericin B that does not contain cholesterol (KASOME 10) by arguing that the use of cholesterol in formulating liposomal amphotericin B such as AmBisome contributes to exacerbation of the
disease. In other words, the authors argue that avoiding the use of the cholesterol “could make this drug more suitable for clearing parasites” (page-6 line-3). Please clarify the justification based on the following points:

a. The scientific background for this argument should be clearly explained and substantiated with published data.

b. The reference cited for the argument (a review by Pucadyil and Chattopadhyay, 2007) does not fully support the statements on the manuscript (page-5 and 6). For example, the review describes that the host cholesterol promotes antigen presentation by infected macrophages.

c. It appears that the authors does not clearly distinguish the role of the host cholesterol and the one coming from the drugs in promoting or inhibiting VL. How does the cholesterol in the drugs contribute to the internalization of the parasite as the drug is administered well after the parasite establishes itself inside macrophages? Please cite a reference (s), if any, supporting the idea that cholesterol in the liposomal amphotericin B contributes to establishment and/or exacerbation of VL.

3. The authors should have shown that the infection was established in all of the groups before treating the mice with the drug. Otherwise, it would be difficult to know if the near zero liver parasite load on one of the treatment groups is due to failure to establish infection or the result of the antileishmanial effect of the drug at the specified dose. Please respond why you did not show whether the infection worked.

4. What was the rationale of using Day-14 post-treatment to test the in vivo toxicity? The authors should show that the protocol they used was in accordance with some sort of standard. Or, they should cite a published protocol.

B) Minor Essential Revisions:

1. Please describe the drug, KALSOME 10, and its formulation in a more detailed manner.

2. Please explain the rationale for selecting two drug doses, 3.5mg/kg and 7.5mg/kg. And, explain why only a double dose of 3.5mg/kg was not tested.

3. The time gap between the two injections with 7.5mg/kg KALSOME 10 was not stated in the manuscript.

4. Page-7 line17: please specify how many times the dilution was performed.

5. Page-8 line-2: “…..ADULT BALB/c mice…” Please write the actual age of the mice.

6. Page-9 line-18 to 20: Please write the actual values in the differences b/n each of the treatment groups and the controls. As seen in the figure, there is difference between 3.5 and 7.5mg/kg doses as compared to the control group. Percent reduction could be a better description of the differences.

7. Page-9 line-21: “…resulted in complete clearance of….”. As seen in the figure, the clearance was not COMPLETE in the spleen as one mouse showed some parasites in the spleen.
8. Please discuss why the difference in the weight of the liver and spleen from treated and untreated mice was non-significant. Why a near complete clearance of the parasite in the spleen and liver did not result in a proportional reduction in the size of these organs?

9. Please explain the possible reasons/mechanisms for the high level of immunomodulation by KALSOME 10? As stated in the manuscript, the other lipid formulations are not able to modulate the immune response to L. donovani in infected mice. Again, inclusion of AmBisome control would have shown us the real difference between the two drugs under this experimental condition.

10. Page-12 line 10 “….Unfortunately these formulations are very costly and REQUIRE SEVERAL DAYS OF HOSPITALIZATION….“ This statement appears to contradict with the current move to the use a SINGLE DOSE AmBisome for the treatment of VL (no need of prolonged hospitalization).

11. Page-13 line-5 and 6: If the authors knew that the drug at the specified doses was safe with “no hepatic and renal functional impairment”, what is the importance of doing liver and kidney function tests in the current study?

12. This study was done to test the efficacy of the drug at a lower dose than previously studied (Mishra et al, 2013). However, the authors followed a different protocol from the one used by Mishra et al, 2013. These include the inoculum size and the stage of the parasite used to establish infection. Would it be possible to compare the results of the two studies?

13. Page-15 line-5 to 13: This was a repeat of what was described in the “results” section. Please delete or re-write it.

14. There are several typos in the manuscript. Please correct them.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests