Reviewer's report

Title: Resistance of L. amazonensis and L. braziliensis to nitric oxide correlates with disease state

Version: 1 Date: 24 July 2006

Reviewer: Silvia Uliana

Reviewer's report:

General

The paper addresses the interesting question of differential susceptibility of Leishmania isolates to nitric oxide (NO).

In vitro susceptibility tests performed in the presence of the NO-donor NaNO2 revealed that promastigotes are killed by very high concentrations of NaNO2. It is not clear whether this concentration range could be achieved in vivo by natural NO production mechanisms. Nevertheless, the study discloses differences in susceptibility between isolates from patients, a very interesting observation.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Background:
Nomenclature: Care should be taken regarding the recommended nomenclature for Leishmania species.
For example, use Leishmania (Viannia) braziliensis (and not Leishmania (L.) viannia braziliensis), L. (V.) guyanensis (and not L. (L.) braziliensis guyanensis), L.(L.) amazonensis, etc. For a detailed description see Lainson and Shaw (1987) in The Leishmaniasis in Biology and Medicine, ed. Peters & Killick-Kendrick.

Methods:
Evaluation of NO susceptibility of Leishmania spp promastigotes by thymidine incorporation. Describe the method thoroughly with number of parasites per volume, exposure to drug (which is not described in the above paragraph), and method for determining thymidine incorporation. The same applies to the topic Colorimetric method for evaluation where description is inconsistent.

It is essential that authors state whether the NaNO2 solutions were freshly prepared just before incubation with the parasites.

Infection of macrophages. A detailed description of experiments with human macrophages should be provided. Were cells pooled from different donors? Were the assays repeated with cells from the same or different donors? Were L. amazonensis and L. braziliensis isolates tested in infection with cells obtained from the same donors?

Results:

Table 1 is missing.

Individual susceptibility data for the 28 isolates must be shown: Figure 1 shows results for 14 isolates in total (8 resistant and 6 susceptible) while 28 isolates were tested. It is not clear why.
On the other hand, the presentation of the results as the means for combined L. braziliensis and L. amazonensis isolates is confusing and does not allow evaluation of possible differences in susceptibility between species, which would be interesting.

Figure 2. In the Results section, the authors state that data on the initial lesion size was available for 12 patients with CL, while Figure 2 shows data for 14 patients.

Discussion

In my opinion, authors should make clear that in vitro susceptibility data reveals that promastigotes are killed
only by very high concentrations of NaNO2 and speculate on the actual relevance of this finding in the intracellular natural environment.

On the other hand, concluding that higher virulence of some isolates (as reflected by increased lesion size) correlates with resistance to NO seems to be too strong a conclusion, given that: 1) sensitivity of amastigotes was not directly tested and 2) production of NO in infected macrophages was not measured. It seems to me that we should not assume that amastigotes and promastigotes are equally sensitive to NO. Also, as production of NO was not measured on macrophages infected with the â€œresistantâ€ and â€œsensitiveâ€ isolates, other mechanisms for greater survival of the resistant isolates can be in place.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Background:
1- As it is, the statement â€œThe role of NO in leishmanicidal activity of human macrophages, however, has been debated. Recent data suggest NO does play a role, but the importance and nature of this role is not clear [18]â€ is not clear and implies that data on ref [18] refers to leishmaniasis. I suggest changing to â€œThe role of NO in leishmanicidal activity of human macrophages, however, has been debated. Recent data suggest NO does play a role in human response to infection, but the importance and nature of this role is not clear [18]."

2- Resistance of M. tuberculosis to NO has been described, but not in ref [22]. That is a review that does not mention specifically resistance of M. tuberculosis to NO. The work by O'Brien et al. Infect Immun. 1994;62(11):5187-90 is probably a better reference.

Methods:
It might be advisable to include Ethical Committee approval to collection of parasites from patients.

Isolation and cultivation of L. braziliensis and L. amazonensis: What was the mean and SD for time of storage of the selected strains?

I suggest moving the â€œEpidemiological and clinical evaluationsâ€ section to the end of Methods, just before â€œStatistical Analysisâ€.

Figures:

Figure 1B: Evaluation of viability by MTT is presented as a function of OD(540nm), with large standard SEM. As OD values for MTT tests are considerably variable from experiment to experiment, comparing the percent survival related to the untreated control in each experiment could be an alternative to that.

Figure 2. Axis titles should be â€œParasites isolated from CL patientsâ€ and â€œLesion size (mm)."

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests:
I declare that I have no competing interests.