Reviewer's report

Title: Microbicide excipients can greatly increase susceptibility to genital herpes transmission in the mouse

Version: 1 Date: 2 June 2010

Reviewer: Deborah Palliser

Reviewer's report:

susceptibility to genital herpes simplex virus-2 (HSV-2).

The work builds on a previous study by the authors (Cone et al, BMC Infectious Diseases, 2006) that used the mouse HSV-2 model to predict toxicity associated with vaginal microbicides.

In the submitted study the authors show convincingly that some excipients increase susceptibility to HSV-2 infection. Significant changes to HSV-2 transmission were observed when mice were treated with KY warming jelly (KYWJ) and its primary constituents, propylene glycol and PEG-8. In addition, the authors were able to detect relatively modest changes in HSV-2 susceptibility in response to some excipients (2-5 fold over controls), provided a large number of animals were used.

However, the study falls short in terms of data presented and alternative explanations for the outcomes observed.

Major Compulsory Revisions.

Background

The citation for Mesquita et al needs to be updated (#14). This was published in JID, 2009.

Results

1. Figure 1 needs to be simplified. It is unclear how inclusion of data from the previous study adds to the figure.

   It seems sufficient to state in the text (which the authors have done) that the curves are almost identical. The arrows in the figure also seem redundant. It is clear from the text, the use of different symbols in the figure and the position of the symbols on the graph, that these represent outliers derived following treatment of mice with the excipients described.

2. From Table 1, susceptibility was highest in mice pre-treated with KYWJ or its constituents: for 5% GML+KYWJ fold increase in susceptibility is reported as #10 and for KYWJ #9.
The authors then go on to treat additional mice with either of these two treatments followed by 0.1 HSV-2 to “obtain a more accurate measure of the magnitudes of the susceptibility increases”. These results are shown in Table 2; 10 fold greater for 5% GML+KYWJ and 7 fold greater for KYWJ alone. The authors also demonstrate that the 5% GML alone, formulated in PBS, also increased HSV-2 infection. These observations are striking, especially taking into account the 2 recent publications by the Haase group that suggest GML could be used as an HIV-1 microbicide (which the authors cite).

However, more data needs to be presented that try to determine how or even whether 5% GML, in the presence or absence of KYWJ is toxic. The HSV-2 infection assay is important and intriguing, but when taking into account the Haase publications and studies that attempt to define biomarkers for assessing microbicide toxicity (including the authors previous studies), there needs to be some experimental data to attempt to define what is causing the increased susceptibility observed. For example, when Schlievert et al (Antimicrob Agents Chemother, 2008) use 5% GML+KYWJ in a macaque they see no evidence of inflammation and observed decreased IL-8 production. It would be useful to know whether chemokines/cytokines are induced either with KYWJ alone, GML alone or 5% GML+KYWJ. In the authors’ 2006 BMCID study IL-1#, IL-1#, KC, MIP-1# and RANTES production are used as a readout for microbicide toxicity. Despite some caveats with respect to kinetics, the authors report that these chemokines are upregulated when mice were treated with candidate detergent microbicides, and could be useful biomarkers for toxicity. As GML is reported to reduce production of these cytokines (Li et al, Nature 2009), this data is extremely important. For GML, reduced levels of cytokines could result in decreased HSV-2 protection as cytokines such as MIP-1#, MIP-1# and RANTES have been implicated in preventing HSV-2 transmission (for example, Tengvall et al, J Virol. 2006).

3. In addition to chemokine/cytokine production, immunohistochemistry of vaginal and cervical sections to determine changes in cellularity would be appropriate.

4. Microscopic analysis to assess any epithelial disruption also needs to be included to confirm increased HSV-2 is due to toxicity rather than, for example, altered chemokine profiles. This would be particularly important for the excipients the authors postulate are altering HSV-2 susceptibility due to hypertonicity.

5. Finally, in their previous publication the authors maintain that assessing HSV-2 infection 3 days following infection is a true reflection of whether an animal becomes infected or not. However, the authors do not report viral titers obtained. Would it not be possible that if viral titers are low, virus may be cleared? Inclusion of viral titers could be useful as an additional parameter of excipient toxicity.
Discussion
1. The discussion is very long, and would benefit from being sub-divided into sections. For example discussion about KYWJ and GML potential mechanism of toxicity could make up one section, advantages and disadvantages of the model could be another.
2. In the discussion of KYWJ and GML, it is noted that both agents “individually mediated susceptibility increasing toxicity”. As both GML in KYWJ and GML in PBS mediate increased HSV-2 susceptibility isn’t it likely that this increased susceptibility is mediated via different mechanisms? The authors postulate hypertonicity for KYWJ, but that would seem unlikely for GML (see point #3).
3. As an extension of point #2, in the discussion of GML, it should be noted that the altered susceptibility to HSV-2 may be due to decreased chemokine production (if this is what the authors observe). If this is the outcome, it could be postulated that the altered susceptibility to HSV-2 observed following GML treatment is due to different reasons than that observed for KYWJ (and other excipients). On the one hand, and as the authors point out in the Background section, this is a strength of this study: irrespective of the mode of action of an excipient or API- as the readout is HSV-2 infection, any detrimental effect the excipient or API has will be detected. However, as requested above, the authors need to show that the increased susceptibility they observe is due to toxicity, for example loss of epithelial integrity, upregulation of inflammatory cytokines, rather than, for example decreased chemokine production.

Minor Essential Revisions.
Results
1. Page 10, line 10-12, add the words neat to the sentence “KYWJ and both of the humectants/solvents in this gel, propylene glycol (neat) and PEG-8 (neat), also caused...” to avoid confusion with the propylene glycol at 10% concentration described in page 10, line 6-7.
Page 10, line 13, add the word neat “animals treated with the (neat) humectants/solvents...”.
Discussion
1. Page 13, line 16, “Moreover, GML is currently being being studied as a...” Remove the word been.

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 I have no competing interests