Reviewer's report

Title: HIV-1 Tat Protein Alter the Tight Junction Integrity and Function of Retinal Pigment Epithelium: an in vitro Study

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Reviewer: Georgette D Kanmogne

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The manuscript by Bai et al, entitled: “HIV-1 Tat Protein Alter the Tight Junction Integrity and Function of Retinal Pigment Epithelium: an in vitro Study” studied the effects of HIV-1 Tat proteins on the barrier function and tight junction protein expression in a retinal epithelial cell line. Below are my comments on the manuscript.

1. Is the question posed by the authors well defined?
The authors stated the study objective in the “background” section of the abstract. There should also state the study rational here.

2. Are the methods appropriate and well described?
Yes, the methods are appropriate and well described.

3. Are the data sound?
The data are sound, but weak and incomplete. This study will be strengthened by additional experiments looking into the mechanisms of Tat-induced dysfunction of brain-retinal barrier. In addition, the authors should explain their reasons for selection the TJs studied (occludin, claudins 1-4). Are other TJs proteins (ZO-1, ZO-2) expressed in those cells? If yes, are they affected by Tat treatment? Do they play a role in Tat-induced dysfunction of brain-retinal barrier?

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?
Yes.

5. Are the discussion and conclusions well balanced and adequately supported by the data?
The discussion is too long and could be shorten: e.g: Page 13, lines 2-6 is further description of the methods and should not be in the discussion. Also, some background literature can be move to the “Introduction – Background” section, and only the study findings discussed in the Discussion section.

6. Are limitations of the work clearly stated?
Limitations of the work are not stated. Example, these studies were performed in a cell line and may not necessary be extrapolated to human cells or in vivo
situation. Confirming the finding using human primary retinal epithelial cells, and/or retinal autopsy tissues from infected and non-infected patients will add significant strength to this study.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
   Yes

8. Do the title and abstract accurately convey what has been found?
   Yes

9. Is the writing acceptable?
   Authors should do spell check and correct the mistakes mentioned below:

   1. Abstract.
      Background: “To determine the effects of HIV-1 Tat proteins on the barrier function and tight-junction protein expression of retinal pigment epithelial cell(RPE)”…
      This appears to be the study objective or aim, not the background.
      Conclusions: “These maybe contribute to the pathogenesis of HIV-related ophthalmopathy.” Should be. “may contribute to…”

   2. Page 4, line 6 “….TER and permeability…”, not “perimeability”
      Is it “fluorescence sodium” or “fluorescence sodium”?

   3. Reagent. Please give the source and molecular weight of fluorescence sodium

   4. Cell viability assay. Please check for symbols and units. Example, 150 ml DMSO is likely inappropriate and is probably 150-ml. There are several examples in the manuscript. This should be checked before and after uploading the manuscript, as the uploading process sometime change / distort the symbols.

   5. Page 5. Measurement of TER.
      Minor comments, there should be a space between number and units (e.g: 0.4 mm; 50 ml, 0.6 cm2).
      Page 6, 3rd and 4th lines. Should “400ul and 600ul” be “400 ml and 600 ml”?
      Line 9…”TER was measured by an epithelial voltohmeter…” should be …”TER was measured with an epithelial voltohmeter…”
      Line 11. “…day10..” should have a space between day and 10.

   6. Page 7. Western blot analysis. Is it “…200 ml of ice-cold lysis buffer…” or “…200 ml of ice-cold lysis buffer…”.
   Page 8, line 5…” (1:500).#:actin…”. Please put a space between “(1:500)” and
“#-actin”.

Page 8: Immunofluorescence microscopy.
Lines 1 and 2. Pleased but a space between “D407” and “cells”; “100” and “nM”.
Lines 7 and 8. “anti-occludin (10 mg/ml), anti-claudin-1 (2 mg/ml), anti-claudin-2 (4 mg/ml),
anti-claudin-3(4 mg/ml), anti-claudin-4(4 mg/ml)”. Pleased check concentrations units for accuracy.

Page 9. TER analysis
“Because the TER appeared to be somewhat affected by the serum, so we reduce the
serum concentration…” Pleased check/correct the sentence structure. Starting
the sentence with “Because”, “so” is not appropriate here.
“…for1 week..” need a space between “for” and “1”.

Page 11. Western blot analysis. Insert spaces to separate words where appropriate.

Discussion:
1. Too long. Do not re-describe methods here, and only discuss the study findings.
2. Check and correct sentence structures. Examples
   a. page 14 “…Certain claudins express high tissue- and cell-type specificities….”.
   b. Page 15, 2nd paragraph… “…Furthermore, it can selective increase the
      paracellular conductivity …”
3. How can expression of claudin-2 disrupt and decrease tightness of the
   epithelial barrier? Has similar observations been made in other studies or in other
   cell types?