Author’s response to reviews

Title: Evaluation of HIV protease and nucleoside reverse transcriptase inhibitors on proliferation, necrosis, apoptosis in intestinal epithelial cells and electrolyte and water transport and epithelial barrier function in mice

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Author’s response to reviews: see over
To: Dr. Rachel Neilan  
*BMC Gastroenterology*  
July, 20th, 2010  
Assistant Scientific Editor

Enclosed is our revised manuscript entitled “Evaluation of protease and nucleoside reverse transcriptase inhibitors on intestinal cell proliferation, necrosis, apoptosis, electrolyte and water transport and epithelial barrier function.” for your consideration for publication in *BMC Gastroenterology Journal* by Manuel B. Braga-Neto, Carolina V. Aguiar, Jamilly G. Maciel, Bruna M.C Oliveira, Jesus E. Sevilleja, Reinaldo B. Oria, Gerly A.C. Brito, Cirle A. Warren, Richard L. Guerrant, Aldo A.M. Lima”, MS: 1723463468354902, with specific revisions as requested in your June 29th, 2010 email with the reviewer’s comments as follows:

**Regarding the first reviewer’s comments:**

Minor Essential Revisions

1) **Please use the correct definition for AIDS also in the abstract**  
   R- We have corrected the abstract accordingly.

Discretionary revisions:

1) **I would still appreciate numerical data on body weight in the manuscript as well as reference to a recent review of ART from a first-tier journal in addition or instead of Refs. 1-4**  
   R- The data on body weight has been added to the manuscript as Figure 1. References 1-4 have been replaced as suggested. Recent manuscripts were added [1-4].
2) I would be helpful if some explanations from the answers to reviewers’ comments would be given in the manuscript.

R- The following explanations have been added to the text:

“Data on nutritional intake was not collected during the time of the experiments. Therefore, we cannot exclude differences in nutritional intake between the groups”. **Methods section page 7**

“Morphologic analyses were carried out by only one investigator in a blind manner; inter or intraindividual variability since measures was not assessed”. **Methods section page 8**

“The proliferation experiments were carried out separately with individual control groups for each experiment, using different cell passage. Therefore, the analyses were done comparing values within each individual experiment”. **Methods section, page 12**

3) Decimal places could be safely reduced for many numbers given in the text.

R- This has been corrected throughout the manuscript.

**Regarding the second reviewer’s comments:**

Minor Essential Revisions:

1) First sentence in the conclusion of the Abstract is confusing. “The PIs, NFV and IDV, increased cell death, water and electrolyte secretion, intestinal permeability and decreased villus length in vivo and cell proliferation and cell death in vitro.” This statement seems to say that cell death was both increased and decreased, plus it is confusing for the reader with regard to which results were in vivo and which were in vitro. Please re-write

R- We have corrected the sentence above as follows:

“The PIs, NFV and IDV, increased cell apoptosis *in vivo*, water and electrolyte secretion and intestinal permeability and decreased villus length and cell proliferation. NFV was the only drug tested that increased cell apoptosis *in vitro*.”

2) In vitro and in vivo are no consistently italicized throughout.

R- We have corrected this throughout the manuscript.

3) Titles and subsections do not have a consistent font of style

R- This has been corrected as suggested.
4) In the Background section; the two halves of the sentence: “Nelfinavir has been demonstrated to induce endoplasmic reticulum (ER) stress, autophagy and apoptosis both in vivo and in vitro and suggested that due to its wide activity, oral bioavailability and familiarity of administration it could be repositioned as an chemotherapy agent [9]” are disconnected and the sentence does not seem to make any sense. Please revise.

R- The sentence has been re-written as follows:

“Nelfinavir has been demonstrated to induce endoplasmic reticulum (ER) stress, autophagy and apoptosis both \textit{in vivo} and \textit{in vitro} and the authors have, therefore, suggested that it could be repositioned as a chemotherapy agent”.

5) Does the ApopTag Plus Peroxidase In Situ Detection Kit actually identify both necrotic cells and apoptotic cells as stated in the Materials and Methods section? Clarify by describing how this works in the M&M, or remove the indication that specific kit was used for “Analysis of apoptosis or necrosis”, and add a description of how necrosis was assayed.

R- “Analysis of apoptosis or necrosis” was changed to “Analysis of cell apoptosis” as recommended. The following was added to the methods section page 8: “The ApopTag Plus Peroxidase In Situ Detection Kit distinguishes apoptosis from necrosis by specifically detecting DNA cleavage and chromatin condensation associated with apoptosis. However, there may be some instances where cells exhibiting necrotic morphology my stain lightly or in rare instance, DNA fragmentation can be absent or incomplete in induced apoptosis. Thus, the results were presented as TUNEL positive cells as recommended [5].”

6) The numbering for figures is out of order with the text. The text jumps from Figure 1 to Figure 6 to Figure 2 to Figure 7 to Figure 3 to Figure 4 and 5. The authors should present the Figures in order.

R- This has been corrected as suggested.
7) The last sentence of the first paragraph in the Discussion needs to be revised. It misleadingly states that: “The results of this study suggest that the selected antiretroviral drugs influence the small intestinal absorptive and secretory functions and the ability of the epithelium to restore itself after injury.” While some absorptive and secretory assays were performed, no experiments were done to test the ability of the epithelium to regenerate itself following injury.
R- This has been corrected as suggested on page 17. The statement “and the ability of the epithelium to restore itself after injury has been removed”.

Discretionary Revisions

1. It was very disappointing to see so many poorly worded sentences, typos, misspelling and inconsistent formatting in this manuscript…too numerous to list, especially considering that this is a “revised” submission. The data from the study and the study itself would undoubtedly be more interesting, seem more relevant, and have a greater impact if the authors spent some quality time on the writing.
R- With apologies, the manuscript has been revised throughout and corrected accordingly.

2. The abstract results section would be just as meaningful and easier to read if it is trimmed down and presented as a summary (i.e. “reduction in the duodenal villus length by 29.9%, 28.4%, 27.3% and 29.5%, respectively” seems to be too much detail for an abstract, and it is unclear what “respectively” means).
R- The abstract has been corrected as suggested.

Reference List


We greatly appreciate your and the reviewer’s compliments and very constructive comments, which have helped us to further strengthen and improve our manuscript.

Sincerely,

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