Author's response to reviews

Title: Dimethyl Sulfoxide Blocks Herpes Simplex Virus-1 Productive Infection In Vitro Acting at Different Stages with Positive Cooperativity. Application of Micro-array Analysis

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Dear Ms Veitch:

We are returning our MS "Dimethyl Sulfoxide Blocks Herpes Simplex Virus-1 Productive Infection In Vitro acting at Different Stages with Positive Cooperativity. Application of Micro-array analysis" by Santiago Aguilar (jsaguila@uci.ed ), Douglas Roy (douglas.roy@ed.ac. uk), Peter Ghazal (pghazal@srv1.med.ed.ac.uk), and Edward K. Wagner (ewagner@uci.ed) that has been revised according to the reviewer's comment as follows:

DISCRETIONARY REVISIONS

2 - Fig1 - y axis should presumably read (PFU x 107) instead of 10-7?

The y axis is PFU x 107. Fig 1 has been corrected accordingly.

3- the authors used DMSO in the study, but did not specify the particular chemical supplier of the DMSO used in the assays, or whether they used DMSO from different manufacturers; this detail may be of interest to readers performing antiviral testing.

We have used DMSO from Sigma and Fisher and we have observed no difference. The sources of DMSO are now indicated in Materials and Methods.

4- Fig Legend to Fig 3 refers to "HDD" cells: these are presumably the human foreskin fibroblasts (HFFs) described in the text on p14/15 and defined in the Methods (p6). HDD sho ld be defined in Legend or text, or corrected to HFF.

Legend to Fig 3 should refer to HFF. It is now corrected.

5- Fig 6 + p13: the authors interpret the expts in Fig.6 as effects on virion stability. In the absence of any biochemical experimentation to substantiate this interpretation, I would suggest that the term virion stability be replaced with virion infectivity, which is a more acc rate
description of what is assayed.

We agree with the reviewer that "virion infectivity" is more accurate than "virion stability" and have made the substations.

6- p14/15: the array analysis was performed with human fibroblast infections using the 4% DMSO concentration determined earlier to be effective at blocking HSV replication (Figs 1+3) with little (20%) effect on cell viability in Vero cells. In switching to HFF cells, the authors did demonstrate that DMSO had an antiviral effect in those cells (1.4% IC50)) comparable to that in Vero cells (2%). However, my concern is that having profiled the antiviral effect in HFFs, they did not furnish any cytotoxicity data in human fibroblasts. I would recommend that such data be provided (if available), as it was for Vero cells (Fig2). As the array work is based on an assumption that the 4% DMSO was non-toxic, and that all changes in viral gene expression are therefore solely due to the antiviral effects of DMSO, it is important to address this point.

We have studied the cytotoxic effect of 4% DMSO on HFF cells and found that an 8 hr treatment cause a cell mortality that is not different from Vero. Some cytotoxicity is observed after 24 h treatment, but the array data are for 6 h treatments. These observations are included in the text of the MS and are consistent with the lack of effect of DMSO on cellular transcript levels in mock infected cells.

7- p18 "all these actions are observed at conditions in which DMSO does not have any significant cytotoxic action"- see (6) above; not clearly addressed in HFF.

This issue has been now addressed. See 6.

Editing suggestions:

We have corrected the spelling as suggested by the reviewer.

SUMMARY OF THE CHANGES:

We have done the following changes to the MS:
1) The abstract has been modified according to the Biomedcentral format. It now contains "Background", "Method", "Results" and "Conclusion" sections.
2) Several modifications have been introduced in the text following the reviewer's recommendations, as described above.
3) The section "Authors' contributions" has been added.
4) The references are now in the Biomedcentral format.
5) Table I is now in a separate file because of its size.
6) Fig. 1 has been modified to correct the mistake detected by the reviewer (See 2-).
We hope that we have made the appropriate corrections and expect that the MS be accepted in its present form.

Sincerely,

J.S. Aguilar, E.K. Wagner