Reviewer's report

Title: Raltegravir decreases inflammatory signaling in brain macrophages in human immunodeficiency virus infection in vitro

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Reviewer: Rick B Meeker

Reviewer's report:

This is a relative straightforward study to assess the neurotoxic potential of the HIV integrase inhibitor raltegravir. Markers of neuronal structure and glial activation are used and cytokine secretion was assessed in microglial cultures. Since the inhibitor penetrates the BBB it may have an important role in the control of CNS HIV production. Thus, it is important to know if there is a risk of adverse effects in the CNS. The measures used are relatively sensitive to neural damage and changes in inflammatory status thereby providing a useful index of potential adverse effects. As noted by the authors, a limitation of the study is the absence of data on the number of cells infected. Also, in most experiments, no dose-effect studies were performed. Although there are some limitations, the data provide a clear indication that raltegravir does not have significant toxic effects using a relatively sensitive in vitro assessment and may have some beneficial anti-inflammatory effects. Several items should be addressed by the authors.

Major

1. Clarification of the statistical analysis would be useful. Equation 1 is not a straightforward linear model as described. Is it the model used for assessment of interaction effects? A more detailed description would help.

2. The changes in TNF in Table 1 are not readily apparent in Figure 2. In addition there appears to be an extra data point on the graph. Based on Table 1, raltegravir could be interpreted as having inflammatory activity due to the increase in TNF.

3. Microglial survival or proliferation could be important factors in the interpretation of the results. Data showing the relative cell density should be provided.

4. The authors should indicate whether the cultures were sampled with complete medium replacement or without replacement. Interpretation of the rate of cytokine production differs depending on the protocol.

Minor

1. On pg 7 Fig 3 should be Fig 4.
2. Since a single dose was used for many of the experiments it would be useful to include the rationale for the selected dose in the methods.

3. Do the references provided for the neuronal culture technique describe the protocol used here?

4. It is very difficult to stain for NFkB and the stain in the nucleus is difficult to see in Fig 3. The unmodified values (before arcsine transform) for % of stain in the nucleus should be provided. Given the lack of significant effects on NFkB except for HIV, which are easily summarized in the text, the authors may want to consider removing this figure.

Discretionary

1. I recommend eliminating Table 2. The statistical results from the analysis of the interactions can be adequately summarized in the text.

**Level of interest:** An article of importance in its field

**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