Reviewer's report

Title: Synergistic action of N-palmitoylethanolamine and the FAAH inhibitor URB597 on melanoma growth

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Reviewer: Virginia S Seybold

Reviewer's report:

This manuscript describes an interaction between PEA and the FAAH inhibitor URB597 in the reduction of tumor cell proliferation. With the exception of the report of the levels of PEA, AEA and 2AG in tumors, the biochemical analyses are carefully conducted. Although a clear treatment effect was observed, there are weaknesses in the design of the pharmacological experiments such that the authors over-interpret the data. Revisions of several conclusions are required.

Major compulsory revisions:

1. The experimental design does not meet the criterion to conclude that PEA and URB597 act synergistically. An isobolographic analysis is necessary to make this conclusion. The data, however, do support a supra-additive effect.

2. The authors miss-use the word “potent”. The author's conclusion that “PEA was equipotent if not more potent than AEA and 2AG in inhibiting tumor growth” is not supported by the data; no concentration-response data were shown. PEA may have had a larger effect at a single concentration (Fig. 1), but a statistically significant difference was not reported. Similarly, the authors conclude that URB597 was most potent in inhibiting PEA hydrolysis when no concentration-response data were shown (p. 18) nor were differences in effects tested.

3. The authors cannot claim that PEA reduced B16 cell viability at a concentration <10 µM if they do not show the data.

4. Methods section 3.1: The authors determined hydrolysis of AEA, PEA, and 2-AG as a measure of the enzymes that degrade the compounds, not as an assay of the compounds themselves. Thus, the introductory sentence needs to be revised.

5. The authors normalized all of the measures of AEA, 2AG and PEA to control values so no raw data are reported. In order to assess the reliability of their measures, actual values for the control group need to be reported. In addition, data for levels of the compounds in tumors would need to be normalized to some other factor in order to control for variability in samples (e.g., sample wet weight, protein, or total lipid weight). This is not described in the methods.

6. The data for the receptor antagonists are not compelling. The experiments used high concentrations of PEA and URB597. What are the data that the
antagonists effectively block the receptors at concentrations relative to those of the agonists? The data would be more convincing if the authors confirmed that receptor selective agonists had no effects at high concentrations. In addition, the antagonist data are difficult to interpret because there are no data for the antagonist alone. At face value, though, the antagonists did not reverse the effect of PEA +/- URB597, but cannabidiol may have enhanced the effect (suppl fig 3). The authors do not address this in the figure legend or the text.

Discretionary revision:

Introduction: P. 4: reference to a cannabimimetic effect of PEA is awkward because "mimetic" effects refer to effectors or receptor mediated events. As acknowledged by the authors, PEA does not bind to CB receptors.

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 that I have no competing interests