Review 1: "SARS-CoV-2 Spike protein promotes hyper-inflammatory response that can be ameliorated by Spike-antagonistic peptide and FDA-approved ER stress and MAP kinase inhibitors in vitro"

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RR:C19 Evidence Scale rating by reviewer:

- **Reliable.** The main study claims are generally justified by its methods and data. The results and conclusions are likely to be similar to the hypothetical ideal study. There are some minor caveats or limitations, but they would/do not change the major claims of the study. The study provides sufficient strength of evidence on its own that its main claims should be considered actionable, with some room for future revision.

Review:

The manuscript entitled "SARS-CoV-2 Spike protein promotes hyper-inflammatory response that can be ameliorated by Spike-antagonistic peptide and FDA-approved ER stress and MAP kinase inhibitors in vitro" by Alan C-Y Hsu et al. has highlighted the therapeutic target (using repurposing existing FDA approved drug) for COVID-19 treatment. The hypothesis has been tested by several biochemical methods with a thoughtful idea. The present article could be strengthened by the following major points:

1. Page 5 line 122: To investigate if SARS-CoV-2 spike protein induces pro-inflammatory cytokine production, the author makes a dose-dependent curve (Fig. 1A-C) for 24 hours. The reviewer is wondering if there is any time-dependent curve. Or, in other words, how did the author pick up the time point for 24 hours?
2. The limitation of the FDA approved drug for COVID-19 treatment.
3. 4-PBA is an ER stress inhibitor. Is there any parallel experiment to show the reverse mechanism of 4PBA using tunicamycin (TM) as it is an ER stress inducer?
4. Figure 5: A part of the mechanism of the present study is the involvement of the ER stress pathway mainly IRE-1 involvement. The author is curious to see if CoV2-S1 has an impact on the downstream molecules of IRE-1 as an activation of IRE-1 induces X-box binding protein 1 (XBP-1) mRNA splicing to generate the active form of the XBP1 transcription factor.
5. The present manuscript has demonstrated the SARS-CoV-2 spike protein promotes inflammatory response which could be improved by spike-antagonistic peptide and FDA- approved ER stress inhibitor. There is a review article that addressed the proposed COVID-19 therapeutic target and the proposed mechanism is ER stress. The reviewer thinks the present article would be strengthened by referring to the article: doi: 10.1016/j.lfs.2020.117842. Epub 2020 May 23.

6. To show the antiviral activities of the proposed peptide, the author has performed overall studies using a single cell line. The reviewer has suggested monitoring the same therapeutic window using an additional cell line if possible.
7. Page 15 line 450: "All procedures were performed according to approval from The University of Newcastle Human Ethics Committees." Please indicate the IRB approval number.
8. Page 15 line 455: Please mention the reference after the sentence of "...accordance with standard guidelines."
9. The author sometimes took the measurements of the required reagent in the experiment by volume. Due to the repetition of the experiment the author needs to mention the actual concentration (unit/volume for example $\mu\text{g/mL}$ or ng/mL) of the reagents not by the volume such as $62.5 \mu\text{L}$ hydrocortisone; *line 461*. What is the stock concentration? The author needs to maintain consistency in the writing. For example, the author has mentioned $4 \mu\text{L}$ of $25 \mu\text{g/mL}$ (*line 460*) whereas in the latter mentioned (*line 466*) by 0.5 ng/mL of rhEGF.
10. The methodology section could be improved by mentioning at least the seeding cell numbers for the cell viability assay, siRNAs, PCR array, immunoblotting, and the immunofluorescence experiment.
11. The author needs to mention the amount of loaded protein for the immunoblotting and immunoprecipitation experiments.
12. The author needs to mention the scale bar and magnification for the immunofluorescence microscopy images.