Review of: "Probenecid inhibits SARS-CoV-2 replication in vivo and in vitro"

Charles PAVIA[1]

1 New York Institute of Technology

Potential competing interests: The author(s) declared that no potential competing interests exist.

The paper by Murray et al. (1) offers new hope in the fight against COVID-19 as part of the search for safe and effective therapeutic agents, given that there are currently limited treatment regimens, having varying degrees of success, that involve the use of remdesivir, dexamethasone, and immune plasma or monoclonal antibodies given alone or in combination with each other. The authors describe a well-designed study showing that the anti-gout/arthritis drug, probenecid, has potent in vitro and in vivo activity against SARS-CoV-2, the etiologic agent of COVID-19. In this regard, an acceptable/established animal-infection model of COVID-19 involving hamsters was used here which showed that probenecid blocked viral replication, and this was consistent with relatively high plasma concentrations of the drug circulating in the animals' bloodstream. Accordingly, consideration should be given to testing this drug in a carefully controlled human clinical trial to evaluate its safety and efficacy in treating patients infected with SARS-Cov-2, especially those in critical condition. Before doing this, however, prior safety testing studies in other animal species needs to be done.

Despite these promising results, there are still a few technical issues that should have been resolved prior to the paper's publication which should have been noticed/pointed out by the original reviewer(s) selected by the journal's editorial staff and these are as follows:

1. In the Abstract, it is stated that probenecid was administered as a "single oral dose" while in the Methods section and in the Figure 2 caption, the animals were treated with the drug via the intraperitoneal (i.p.) route. Also, along these lines, i.p. would not be the method of delivery in humans, thus the significance of the results following i.p. injection has to be interpreted with caution, although the overall results showing the antiviral activity of probenecid are highly encouraging.
2. In the Methods section, the number and age of the hamsters used in both the control and test group should have been stated; this is especially important in light of the age difference in the response pattern of young versus elderly people to infection with this virus. Related to this concern, a paper (2) published a year ago showed that, in a modified mouse model of COVID-19, more severe disease occurred in aged mice.
3. It is unclear why only male hamsters were used in this study. Although the hormonal influence and possibly other factors during virus infection for female hamsters (if they had been included in this study) may not be comparable to what might occur in humans, it is nonetheless worth considering to

evaluate this parameter.

4. Lastly, it isn't stated in the Methods section how the animals were humanely euthanized. This might be important to know if other investigators wanted to pursue similar studies in hamsters.

Since the above-cited missing pieces of information are lacking in this report, it can only be assumed that the original reviewer(s) of this paper did not inquire or notice that these important details were missing or necessary to be included in the original publication, but this reviewer takes a different point of view. Assuming the authors will be given the opportunity to respond to this subsequent analysis of their paper, it would be helpful if they would provide more follow-up information in their response.

References

1. Murray, J., et al. *Probenecid inhibits SARS-Cov-2 replication in vivo and in vitro*. Scientific Reports, 2021. https://www.nature.com/articles/s41598-021-97658-w.
2. Dinnon, K.H., et al. *A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures*. Nature, 2020. **586**: p. 560-66. https://www.nature.com/articles/s41586-020-2708-8.