

Reply letter to: Inhibition of SARS-CoV-2 replication using calcineurin inhibitors: Are concentrations required clinically achievable?

Dear Editor,

In response to the letter by Dr. Solanich and col., we acknowledge the authors for their comment about our recently published paper in *Journal of Internal Medicine* [1], on Cyclosporine plus steroids for COVID-19 treatment. Dr. Solanich and col. argue that it is unlikely that an *in vivo* antiviral effect of cyclosporine A (CsA) participates on the beneficial effects observed in our study on patients with COVID-19; their argument is based on a published paper showing that an *in vitro* concentration of 3.3 μM of CsA is necessary to reach LD50 of antiviral activity against SARS-CoV-2 tested on Vero cell cultures [2], which would be equivalent to 3968 ng/ml in plasma, representing 10 times the recommended clinical safe levels of CsA. Then, since in our clinical pilot study we used CsA at a maximum of 2 mg CsA/kg, this dose would mean that the plasma levels reached are far from the 3968 ng/ml needed to get the *in vitro* concentration that shows antiviral activity. Therefore, we agree that the improvement observed in our study in patients with COVID-19 may be due mainly to the immunomodulatory effect of CsA, mostly in patients with moderate-to-severe disease.

In general, extrapolating the *in vitro* results of static drug testing, to the dynamic *in vivo* interactions in humans, is a complex issue [3]. Even across *in vitro* studies, results are difficult to compare because of the use of distinct cell line models or even human pulmonary explants [4], as well as different virus strains, a multiplicity of infection, timing and methods to assess the antiviral activity. For example, Pfefferle and cols. determined differently *in vitro* LD50 for CsA testing across several coronaviruses [2], whilst other studies showed that even higher [5] or lower [6] concentrations of CsA were needed to observe antiviral effects against coronaviruses, tested upon distinct experimental conditions. We do not know a specific study testing *in vitro*, the antiviral CsA activity against SARS-CoV-2.

Since we know neither the actual SARS-CoV-2 viral load in these COVID-19 patients nor the differences in viral load across tissues and cell types, we think that when used at the recommended clinical doses, an antiviral effect of CsA or tacrolimus could not be ruled out. Interestingly, and unexpectedly, a recent study in the UK showed that SARS-CoV-2 infections were less frequent in transplanted patients receiving immunosuppressive therapy (mainly tacrolimus, cyclosporine and steroids) compared with those on a transplant waiting list, with no immunosuppression [7]. Similarly, an international, multicentric study showed that only 144 out of 9845 patients with kidney transplants and immunosuppressive therapy were hospitalized with COVID-19, and most of them, with comorbidities [8], suggest possible protection by tacrolimus or CsA, acting upon undetermined mechanisms.

In conclusion, although our study was not designed to prove that the benefit from CsA is the consequence of an antiviral effect and we only suggested this as a possibility, such effect cannot be ruled out, and further research to test such hypothesis is warranted.

Conflicts of interest

The authors declare they have no conflicts of interest.

J. L. Gálvez-Romero¹ ; J. Deveaux-Homs²; F. A. Real-Ramírez³; O. Palmeros-Rojas⁴  & S. Pedraza-Sánchez^{5,6} 

From the ¹Departamento de Investigación; ²Dirección Médica; ³Departamento de Medicina Interna, Hospital Regional Puebla del ISSSTE, Puebla; ⁴Área de Matemáticas, Universidad Autónoma de Chapingo, Texcoco; ⁵Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMSZ), Tlalpan; and ⁶Facultad de Ciencias, Universidad Nacional Autónoma de México (UNAM), Coyoacan, México

References

- 1 Gálvez-Romero JL, Palmeros-Rojas O, Real-Ramírez FA, Sánchez-Romero S, Tome-Maxil R, Ramírez-Sandoval MP,

- et al. Cyclosporine A plus low-dose steroid treatment in COVID-19 improves clinical outcomes in patients with moderate to severe disease: A pilot study. *J Intern Med.* 2020. <https://doi.org/10.1111/joim.13223>.
- 2 Pfefferle S, Schopf J, Kogl M, Friedel CC, Müller MA, Carbajo-Lozoya J, et al. The SARS-coronavirus-host interactome: identification of cyclophilins as target for pan-coronavirus inhibitors. *PLoS Pathog.* 2011;**7**:e1002331.
 - 3 Lin Z, Zhou D, Hoag S, Qiu Y. Influence of drug properties and formulation on in vitro drug release and biowaiver regulation of oral extended release dosage forms. *AAPS J.* 2016;**18**:333–45.
 - 4 Li HS, Kuok DIT, Cheung MC, Ng MMT, Ng KC, Hui KPY, et al. Effect of interferon alpha and cyclosporine treatment separately and in combination on Middle East Respiratory Syndrome Coronavirus (MERS-CoV) replication in a human in-vitro and ex-vivo culture model. *Antiviral Res.* 2018;**155**:89–96.
 - 5 de Wilde AH, Raj VS, Oudshoorn D, Bestebroer TM, van Nieuwkoop S, Limpens RWAL, et al. MERS-coronavirus replication induces severe in vitro cytopathology and is strongly inhibited by cyclosporin A or interferon-alpha treatment. *J Gen Virol.* 2013;**94**:1749–60.
 - 6 Ma-Lauer Y, Zheng Y, Malesevic M, von Brunn B, Fischer G, von Brunn A. Influences of cyclosporin A and non-immunosuppressive derivatives on cellular cyclophilins and viral nucleocapsid protein during human coronavirus 229E replication. *Antiviral Res.* 2020;**173**:104620.
 - 7 Mohamed IH, Chowdary PB, Shetty S, Sammartino C, Sivaprakasam R, Lindsey B, et al. Outcomes of renal transplant recipients With SARS-CoV-2 infection in the eye of the storm: a comparative study with waitlisted patients. *Transplantation.* 2021;**105**:115–20.
 - 8 Cravedi P, Mothi SS, Azzi Y, Haverly M, Farouk SS, Pérez-Sáez MJ, et al. COVID-19 and kidney transplantation: Results from the TANGO international transplant consortium. *Am J Transplant.* 2020;**20**:3140–8.

Correspondence: Jose Luis Gálvez-Romero, MD, Hospital Regional ISSSTE Puebla, 14 Sur 4336, Col Jardines de San Manuel, Puebla 72570, México.
(e-mail: jose.galvez@issste.gob.mx) ■