0475

# CETYLPYRIDINIUM CHLORIDE MOUTHWASHES TO REDUCE THE SHEDDING OF VIABLE SARS-COV-2

**J. Muñoz-Basagoiti**[1]*, A. Alemany[2,3,4]*, D. Perez-Zsolt[1], D. Ouchi[1,5], D. Raïch-Regué[1], B. Trinité[1], E. Pradenas[1], J. Blanco[1,6,7], R. León[8], V. Blanc[8], J. Gispert[8], B. Clotet[1,2,3,6,7], O. Mitjà[2,3,7,9&], N. Izquierdo-Useros[1,7,&], CPC Study Group

*Equal contribution [&]Senior authors [1]IrsiCaixa AIDS Research Institute, 08916, Badalona, Spain [2]Fight AIDS and Infectious Diseases Foundation, Badalona, Spain [3]Hospital Universitari Germans Trias i Pujol, Badalona, Spain [4]Facultat de Medicina-Universitat de Barcelona, Barcelona, Spain [5]Universitat Autònoma de Barcelona, Barcelona, Spain [6]Universitat de Vic-Universitat Central de Catalunya (UVIC-UCC), Vic, Spain [7]Germans Trias i Pujol Research Institute, 08916, Badalona, Spain [8]DENTAID Research Center, Cerdanyola del Vallès, Spain [9]Lihir Medical Centre, International SOS, Lihir Island, Papua New Guinea

## BACKGROUND

The airbone transmission of SARS-CoV-2 via respiratory fluids suggests that mouthwashes containing virucidal substances may be good candidates to help reduce viral load in the oral cavity and viral transmission. We hypothesize that cetylpiridinium chloride (CPC), a quaternary ammonium compound present in many mouthwashes with broad antiseptic properties, may have a virucidal effect on SARS-CoV-2.

## METHODS

We tested the capacity of CPC-containing mouthwashes to inhibit SARS-CoV-2 entry into target cells by using a luciferase-based assay with a reporter lentivirus pseudotyped with the SARS-CoV-2 spike protein. The replication-competent SARS-CoV-2 B.1.1.7 and D614G variants were also assayed. CPC's virucidal effect was measured by RT-PCR, by calculating the CPC-treated and non-treated virus tissue culture infectious dose 50% ($TCID_{50}$/mL) and by dynamic light-scattering analyses (DSL). We confirmed the disruption of the viral membrane by using a modified ELISA, which detected the SARS-CoV-2 nucleocapsid (NC) protein in the absence of its own lysis buffer upon CPC treatment. The effect of CPC in the saliva of individuals with CoVID-19 was assessed in a clinical trial (**Fig. 1**).

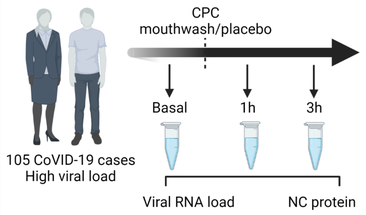


***Fig. 1. Schematic representation of the study design.***

## RESULTS

CPC abrogated SARS-CoV-2 entry into 293T-ACE2-TMPRSS2 cells and had a 50% maximal inhibitory concentration ($IC_{50}$) of 0.47µM (**Fig. 2A**). SARS-CoV-2 treatment for 1-2 min. with a commercial mouthwash containing 0.07% CPC showed reduced infectivity of replication-competent B.1.1.7 and D614G SARS-CoV-2 variants (**Fig. 2B**) but no differences in viral RNA load (**Fig. 2C**).

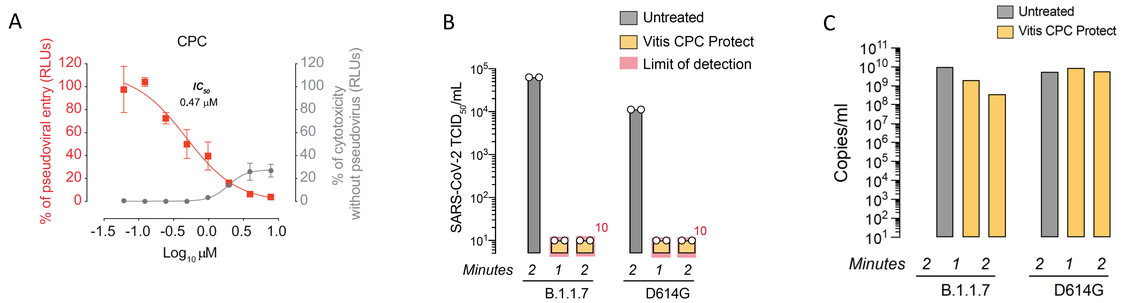


***Fig. 2. CPC has an antiviral effect on SARS-CoV-2.*** *(A) Antiviral activity of CPC against SARS-CoV-2 pseudoviral entry. B.1.1.7 and D614G variants of SARS-CoV-2 (B) infectivity and (C) viral RNA load of non-treated and CPC-treated samples.*

Without the lysis buffer, a NC protein ELISA (**Fig. 3A**) showed a higher SARS-CoV-2 NC detection when virions were treated with CPC (**Fig. 3B**). Moreover, the CPC-treatment broadened the viral size distribution of SARS-CoV-2 variants particles, as detected by DLS analyses (**Fig. 3C-D**).

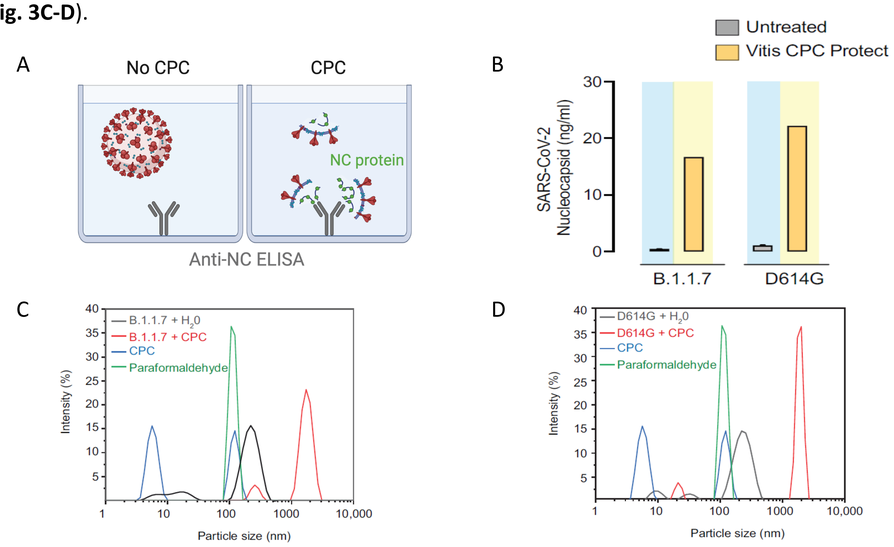


***Fig. 3. CPC reduces SARS-CoV-2 infectivity by breaking the viral membrane.*** *(A) Schematic representation of the SARS-CoV-2 NC protein ELISA performed without its lysis buffer. (B) NC protein amounts (ng/mL) of SARS-CoV-2 B.1.1.7 and D614G variants after a 2 minute CPC-treatment. Hydrodynamic size of SARS-CoV-2 (C) B1.1.7 and (D) D614G variants in the presence of CPC by DLS.*

The assessment of the viral load on 105 patients showed no significant differences by RT-PCR between groups at the different time points (**Fig. 4A**). However, the levels of SARS-CoV-2 NC of lysed viruses were significantly higher in the CPC group compared with the control group at 1 hour (269.3 pg/mL) and at 3 hours post-intervention (561.1 pg/mL) (**Fig. 4B**).

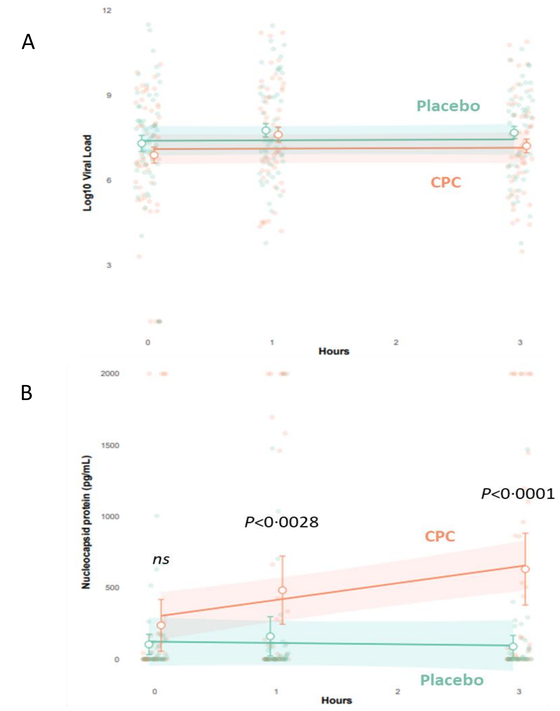


***Fig. 4. CPC-containing mouthwashes disrupt SARS-CoV-2 viral membranes in saliva from CoVID-19 patients.*** *Fitted linear mixed model for change in (A) RNA viral load and (B) NC protein levels. Green line = control group, orange line = CPC group.*

## CONCLUSIONS

Here we show that CPC decreases more than a 1000 times the infectivity of SARS-CoV-2 *in vitro* against different SARS-CoV-2 variants.

In CoVID-19 patients, the use of a 0.07% CPC mouthwash correlated with a statistically significant increase of NC protein levels in saliva, indicating enhanced disruption of viral particles.

**CPC-containing mouth rinses can represent a cost-effective measure to reduce SARS-CoV-2 infectivity in saliva, aiding to reduce viral transmission from infected individuals regardless of the variants they are infected with.**

**ACKNOWLEDGEMENTS**

This research was funded by Dentaid SL. The authors also acknowledge the crowdfunding initiative #Yomecorono. N. I-U. is supported by grant PID2020- 17145RB-I00 from the Spanish Ministry of Science and Innovation. E. Pradenas is supported by a doctoral grant from the National Agency for Research and Development of Chile (ANID 72180406).

IrsiCaixa Institut de Recerca de la Sida · DENTAID RESEARCH CENTER · Germans Trias i Pujol Hospital · FUNDACIÓ LLUITA CONTRA LA SIDA I LES MALALTIES INFECCIOSES