Inhalable Cyclosporine Powder for The Prevention of Pulmonary Rejection and the Treatment of Covid-19

Davide D'Angelo[1], Eride Quarta[1], Stefania Glieca[1], Giada Varacca[1], Martina Brandolini[2], Lisa Flammini[1], Simona Bertoni[1], Vittorio Sambri[2], Fabio Sonvico[1], Ruggero Bettini[1], Francesca Buttini[1]

[1]Food and Drug Department, University of Parma, Parco Area delle Scienze 27a, 43124, Parma, Italy.

[2]Microbiology Unit, The Great Romagna Area Hub Laboratory, 47522 Pievesestina, Cesena, Italy.

davide.dangelo@unipr.it

Background and Aim of the work

Cyclosporine A (CsA) is a cyclic undecapeptide with a powerful immunosuppressive activity. One of the most frequent side effects after lung transplantation is the development of **bronchiolitis obliterans** (BO), considered a marker of chronic rejection, which causes 30% of deaths. Local administration of CsA to the lung could increase the therapeutic efficacy of this molecule in preventing transplant rejection [1]. Alongside this effect, cyclosporine could mediate the containment of the cytokine storm due to **SARS-CoV-2** infection [2]. The **aim** of this project was the production and characterization of a respirable powder containing Cyclosporine A for the prevention of BOS and the inhibition of SARS-CoV-2 replication**.**

Methods

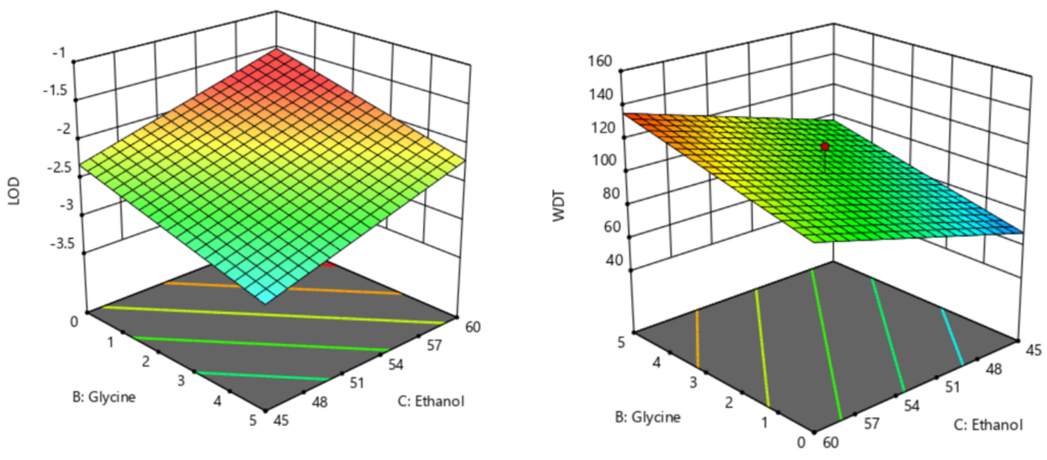
The spray dried CsA powders were obtained starting from a solution 1% (w/v) of water and ethanol. Powders were produced according to a design of experiment with three factors and two levels: Mannitol (20-10%), glycine (5-0%), and ethanol (45-60%).

The dissolution was studied upon powder aerosolization using **Respicell™** apparatus. Dissolution rate was studied using Weibull equation and defined as the time to dissolve the 63.2% of the API. Loss on drying (LOD) was studied by thermogravimetric analysis. The respirability of the best performing powder composed by CsA and mannitol with a ratio of 80:20 (w/w) and 45% (v/v) of ethanol was assessed using Next Generation Impactor (Copley, UK) and RS01® inhaler (Plastiape, Italy). Each HPMC capsule was filled with 20 mg of powder.

Anti-inflammatory effect was studied on THP-1/A549 cell lines co-colture. In vitro inhibition of **SARS-CoV-2** replication was studied on Vero E6 cell line.

Results

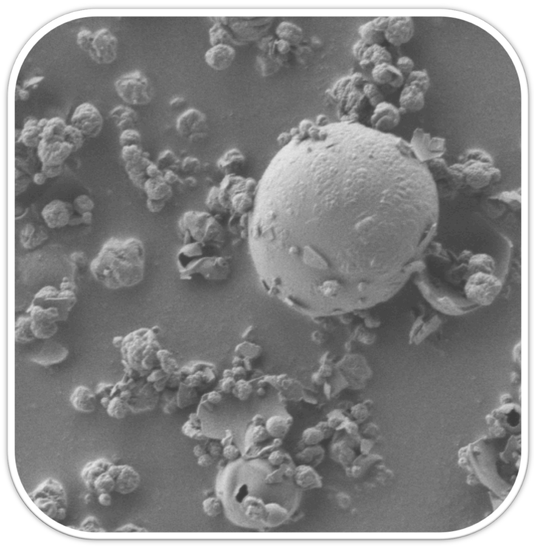
Design of experiment



Graphical representation of glycine and ethanol effect on and loss on drying (LOD) at mannitol concentration of 15% (w / w). Weibull dissolution time (WDT)

ANOVA revealed significant models for the loss on drying (LOD) and Weibull dissolution time (WDT). Ethanol is the main factor influencing the different degrees of residual solvents in the particles. As the percentage of ethanol increases, the LOD value approaches zero per cent. On the contrary, glycine increased the LOD value. Ethanol and glycine also played a significant role in the dissolution time of the SD powders. The effect reported by the model as "positive" is to be meant as an increased WDT value. Therefore, the time to dissolve 63.2% of API increase along with the percentage of ethanol and glycine.

Powder morphology and respirability

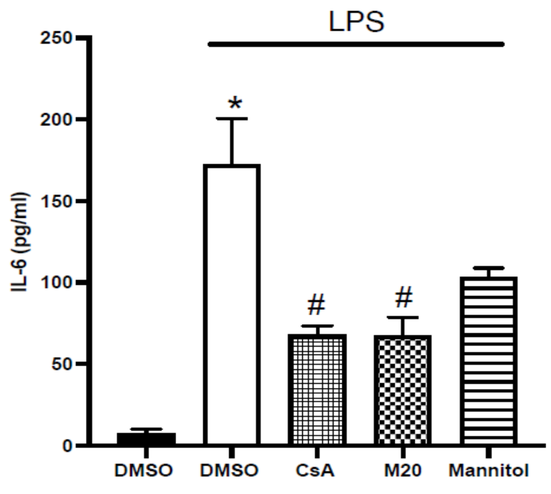


SEM of the powder M20 produced by spray drying.

	Mean (n=3)
Emitted powder (mg)	16.0± 1.69
Emitted CsA Dose (mg)	12.2± 1.57
MMAD (µm)	**1.38± 0.82**
FPD (mg)	10.18± 1.49
FPF <5 µm (%)	**83.28± 0.69**

Next Generation Impactor analysis results. FPD= Fine Particle Dose. FPF=Fine Particle Fraction (calculated on emitted dose). MMAD= mass median aerodynamic diameter.

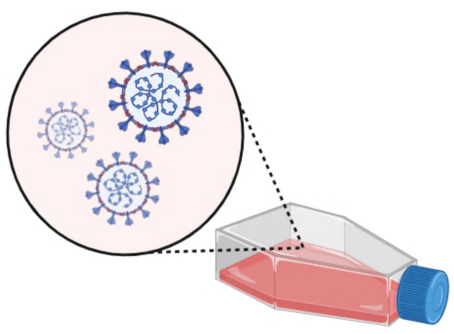
Anti-inflammatory effect

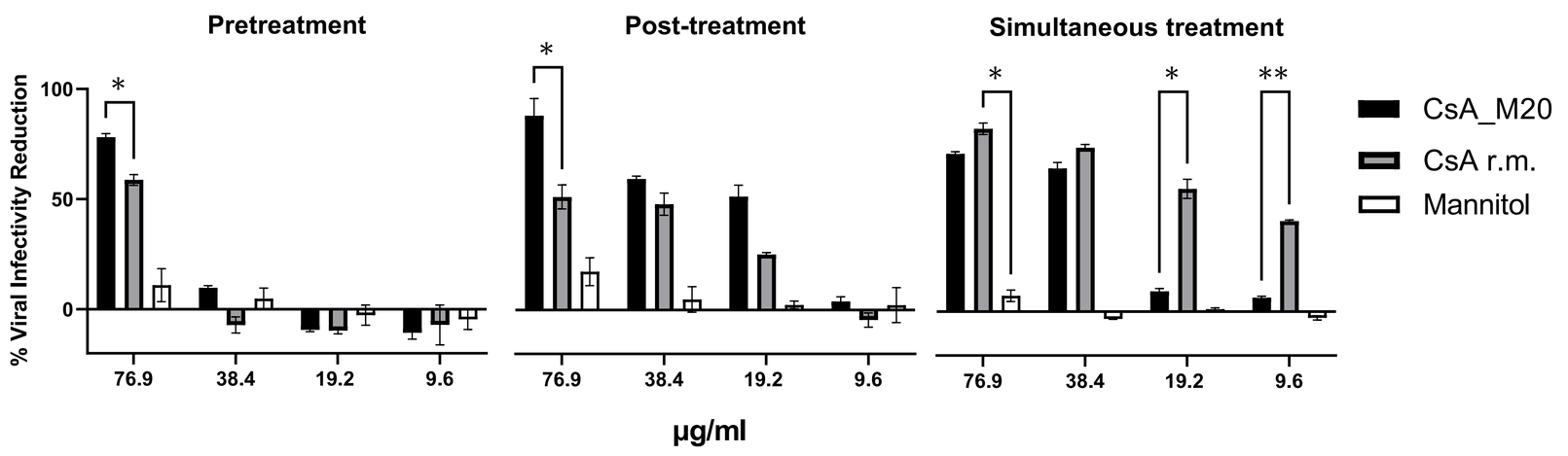


IL-6 ELISA Test, on THP-1 and A549 co-culture. CsA raw material and M20 = 10 µg/mL; LPS= 1 µg/mL.

Key result:
CsA maintained its anti-inflammatory activity after spray drying process.

In Vitro antiviral efficacy on SARS-CoV-2 Omicron BA.1





Key result:
In the two hours post-treatment, the effect of the M20 reached 93% at the highest concentration tested 76 µg/ml) and was significantly superior to the CsA raw.

Viral concentration 0.0005 m.o.i.; Cell line: Vero E6; Pretreatment: one hour before infection; Post-treatment: two hours post infection; Samples suspended in PBS. *P <0.05 vs CsA raw.

Conclusions

A CsA dry powder formulation was efficiently produced by spray drying, using 20% of mannitol as excipient. This powder demonstrated good aerodynamic characteristics and a higher dissolution profile compared to raw material CsA. M20 powder showed a significant anti-inflammatory effect and **inhibition of SARS-CoV-2 replication.** The availability of a CsA pulmonary product in form of dry powder inhaler represents a promising new drug delivery mechanism in **lung transplantation** recipients and for the **containment of the inflammatory process due to SARS-CoV-2 infection**.

References:

[1] Iacono, A. et al. ERJ Open Res. 5, 00167–02019 (2019). [2] Molyvdas, A. et al. Eur. Respir. J. 56, 2002484 (2020).