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SHORT REPORT

Effect of cyclosporin A on respiratory viral replication in fully differentiated ex vivo human airway epithelia

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Abstract

Cyclosporin A (CsA), an immunosuppressive drug used in transplant recipients, inhibits graft rejection by binding to cyclophilins and competitively inhibiting calcineurin. While concerns about respiratory infections in immunosuppressed patients exist, contradictory data emerged during the COVID-19 pandemic, prompting investigations into CsA's impact on viral infections. This study explores CsA's antiviral effects on SARS-CoV-2 Omicron BA.1, Delta variants, and human parainfluenza virus 3 (HPIV3) using an ex vivo model of human airway epithelium (HAE). CsA exhibited a dose-dependent antiviral effect against the SARS-CoV-2 Delta variant, reducing viral load over 10 days. However, no significant impact was observed against SARS-CoV-2 Omicron or HPIV3, indicating a virus-specific effect. At high concentrations, CsA was associated with an increase of IL-8 and a decrease of IFNλ expression in infected and noninfected HAE. This study highlights the complexity of CsA's antiviral mechanisms, more likely involving intricate inflammatory pathways and interactions with specific viral proteins. The research provides novel insights into CsA's effects on respiratory viruses, emphasizing the need for understanding drug–virus interactions in optimizing therapeutic approaches for transplant recipients and advancing knowledge on immunosuppressive treatments' implications on respiratory viral infections. Limitations include the model's inability to assess T lymphocyte activation, suggesting the necessity for further comprehensive studies to decipher the intricate dynamics of immunosuppressive treatments on respiratory viral infections.

KEYWORDS
Calcineurin inhibitor, HPIV3, Immunosuppressive treatments, SARS-CoV-2, Transplantation

Abbreviations: CsA, Cyclosporin A; CyP, Cyclophilins; CyP A, Cyclophilin A; DPI, Day(s) post infection; HAE, Human Airway Epithelium; HPIV3, Human parainfluenza virus 3; HSCT, Hematopoietic stem cell transplant; IFN, interferon; IL, Interleukin; NFAT, Nuclear factor of activated T; PPi, Peptidyl-prolyl isomerases; RSV, Respiratory syncytial virus; RT-qPCR, Reverse transcription quantitative PCR; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.

Cyclosporin A (CsA) is an immunosuppressive drug that prevents graft rejection posttransplantation by binding to cyclophilins (CyP) which are ubiquitous peptidyl-prolyl isomerases (PPi) with various physiological functions. This drug–receptor complex specifically and competitively inhibits calcineurin, a complex of calcium-dependent phosphatases involved in a wide range of cellular processes, including T cell activation. CsA, usually combined with other immunosuppressive drugs, is used in both solid organ transplant and in hematopoietic stem cell transplant (HSCT) recipients to prevent graft rejection. The recommended CsA dosage in post-solid organ transplantation is 3–5 mg/kg/day to achieve a target serum concentration of approximately 0.3 μM. The immunosuppressive nature of CsA raises concerns about the risk of severe respiratory infections, such as influenza virus, respiratory syncytial virus (RSV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and data are contradictory for transplant recipients on immunosuppressive treatments.[1] Early guidelines during the COVID 19 pandemic suggested reducing or discontinuing CsA in transplant recipients to improve infection control.[2] Despite its immunosuppressive effects, recent research indicates that CsA may not be associated with an unfavorable course of COVID-19.[3]

Interestingly, CsA has demonstrated antiviral effects against various viruses, including human immunodeficiency virus, hepatitis C, RSV, and even SARS-CoV-2, albeit at concentrations above clinical therapeutic targets.[4,5] The mechanisms of CsA's antiviral action are complex and may involve calcineurin inhibition, cyclophilin binding, and interference with viral replication pathways.[6] The airway epithelium acts as a frontline defense against respiratory viruses, not only as a physical barrier and through the mucociliary apparatus but also through its immunological functions. It initiates multiple innate and adaptive immune mechanisms which are crucial for efficient antiviral responses. To the best of our knowledge, there are no data about a possible action of CsA on HPIV3, a pathogen frequently involved in respiratory complications following transplantation.[7] Concerning SARS-CoV-2, the data are scarce and restricted to treatments with nonphysiological concentration of CsA. Sauerhering et al. have shown antiviral effects of CsA at high concentration (10 μM) in vitro-reconstituted human airway epithelium (HAE) and in mice (50 mg/kg/day) against one of the first variant of SARS-CoV-2 (isolate BetaCoV/Munich/BavPat1/2020).[8] Similarly, D'angelo et al. have demonstrated antiviral activity of a high concentration (27 μM) of dry CsA inhalation powder against Omicron BA.1 in cell lines.[5]

HAEs derived from bronchial specimens recapitulate many properties of the in vivo airway epithelium, highlighting their potential for the study of biological pathways and therapeutic interventions.[9] Here, we investigated, in an ex vivo model of HAE cultured at the air–liquid interface derived from bronchial epithelial cells of healthy donors[9], the effect of two concentrations of CsA, that is, the high concentration range (30 μM) tested in previous reports[5,8] and the therapeutic concentration (0.3 μM) used in transplant recipients[10] against SARS-CoV-2 Omicron BA.1 and Delta variants and against HPIV3. Each concentration of CsA (the therapeutic concentration used in transplant recipients 0.3 μM and the high concentration tested in previous reports 30 μM[5]) (MedChem HY-B05789) was added daily in the basal medium of the tissue to mimic systemic drug delivery from 3 days before viral infection until 10 days after infection. The absence of CsA cytotoxicity was confirmed using resazurin assay (a fluorometric method measuring cellular metabolic activity) (Figure 1A). The induction of two main cytokines [Interleukin-8 (IL-8) and interferon lambda (IFNλ)] by CsA treatment in absence or presence of viral infection was also assessed by reverse transcription quantitative PCR (RT-qPCR) using SYBR Green. Cell lysates of infected and noninfected HAE were taken at 4 days post infection (DPI) in the presence or absence of treatment with CsA and RNA was extracted (E.Z.N.A viral RNA kit I, Omega, R687402). Quantification of IL-8 and IFNλ was done using specific primers from respectively invitrogen® and mycrosynth® (Fwd 5′GCCTGCTGCAGAGCAGAGAT and 5′TTGGCAGCCTTCCTGATTTC, rev 5′GCTCCAGCGAGCGGTAGTG and 5′AACTTCTCCACAACCTCTG) with normalization to RNAseP housekeeping gene and the use of comparative ΔΔCt method.

In order to obtain similar replication kinetics so that the viruses could be compared with each other, infections were done with a MOI of ≈0.1 and 0.035 for SARS-CoV-2 Omicron BA.1 (GISAID accession ID: EPI_ISL_7605546), Delta (GISAID accession ID: EPI_ISL_1811202) variants, respectively (kindly provided by the Center for Emerging Viruses from the HUG), and a MOI of 500 for HPIV3 (ATCC strain [MK-9 HPIV3]). Tissues were apically inoculated with the virus to mimic the natural respiratory transmission route. Samples were collected daily from the apical surfaces of both CsA-treated and control tissues. Viral loads were quantified by RT-qPCR to assess inoculation leftover (remaining after three wash steps, 2–4 h after inoculation) and subsequent viral replication over 10 days.

We observed an antiviral effect against the SARS-CoV-2 Delta variant, with a reduction in viral load from day 2 to day 10 when exposed to the highest CsA concentration (30 μM), as depicted in Figure 1B. Conversely, regardless of the CsA concentration, viral loads for the SARS-CoV-2 Omicron variant and HPIV3 showed no significant differences compared to controls (Figure 1B,C). When administrated at therapeutic CsA concentration (0.3 μM), the viral load of both SARS-CoV-2 variants remained unchanged in both CsA-treated HAE and control tissues. Results of intracellular RNA quantification highlighted a modification of cytokines expression 4 days postinfection upon CsA treatment with the highest nontherapeutic concentration (30 μM). We observed a statistically significant increase of IL-8 mRNA levels in noninfected HAE and in HAE infected with SARS-CoV-2 (Figure 1E). In contrast, the same CsA concentration resulted in a decreased IFNλ induction in all infected and noninfected HAE (Figure 1F). The therapeutic concentration of CsA (0.3 μM) did not significantly impact IL-8 and IFNλ expression except for IFNλ in noninfected HAE.

By using HAE derived from three different healthy donors, we thus demonstrated for the first time that CsA exhibits a concentration-dependent antiviral effect against SARS-CoV-2 Delta variant. These data not only validate but also extend the observations previously made in cell lines infected with SARS-CoV-2

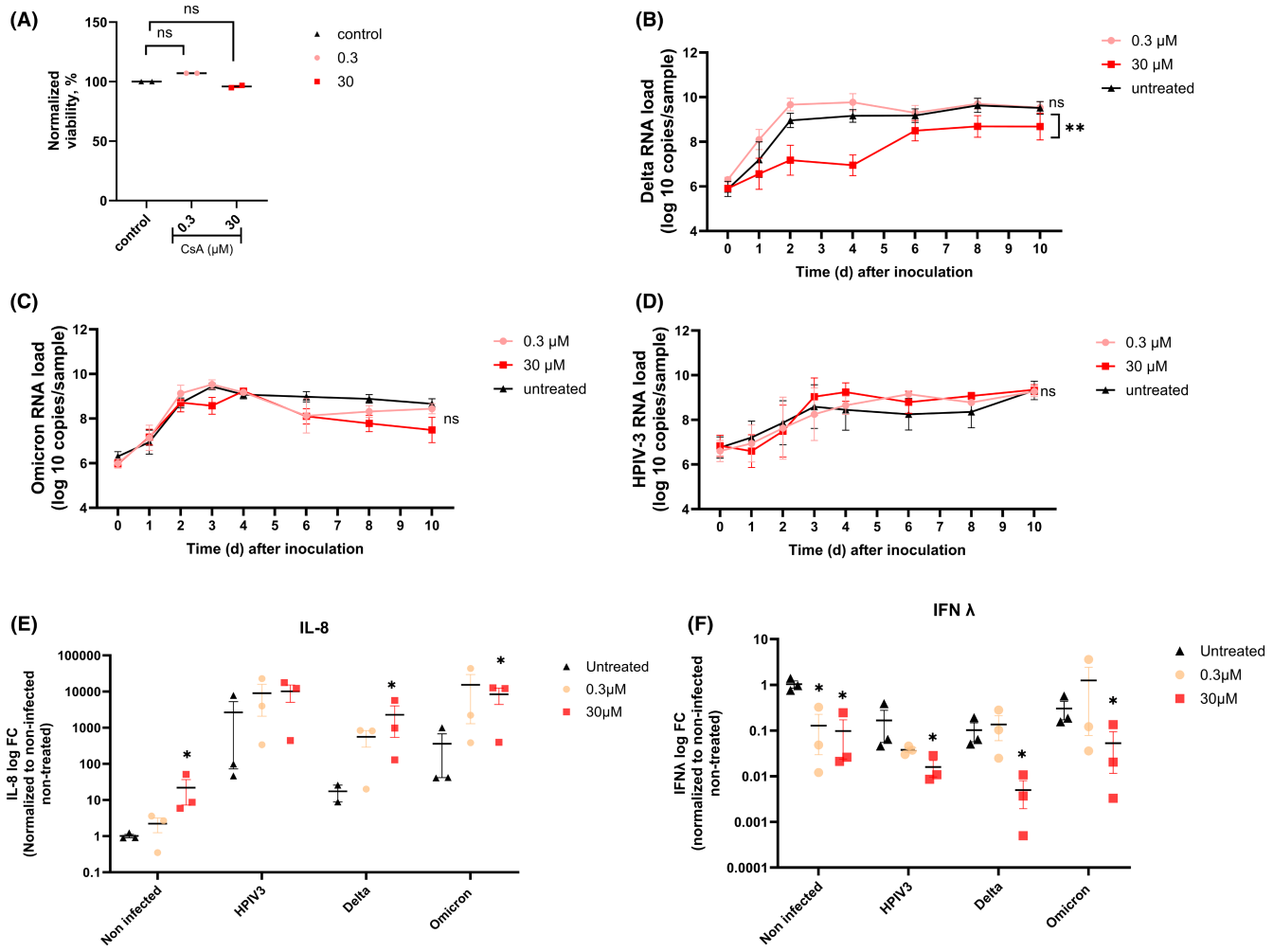

FIGURE 1 Effects of CsA on HAE cell metabolism (A), viral load of SARS-CoV-2 delta (B), Omicron (C) and HPIV3 (D) and on IL-8 (E) and IFNλ (F) intracellular cytokines induction. (A) Illustrates the effects of CsA at day 10 on HAE cell metabolism as measured by resazurin assay, a fluorometric method that estimates the number of viable cells by measuring the reduction of resazurin into resorufin. At day 10 post daily-basal treatment, the cell metabolism was not affected in presence of two different cyclosporin concentrations (0.3 and 30 μM) compared to untreated controls, reflecting the integrity of epithelial cells. The toxicity was tested in duplicate for each concentration and error bars represent SEM. Technical replicates were used to ensure the reliability of single values. Statistical significance was calculated with the one-way ANOVA followed by Dunnett's multiple comparison test using GraphPad Prism 10.0.1. ns: Nonsignificant. HAE were pretreated daily at the basal side, starting 3 days before infection, with two concentrations of CsA (0.3 μM: Pink circle, and 30 μM: Red square). After viral infection of both CsA-treated HAE and HAE controls, apically released viruses were measured and compared to untreated tissues (black triangle). Viral RNA loads of SARS-CoV-2 Delta (B), Omicron (C) variants, and HPIV3 (D) were assessed in HAE from three different donors treated or not with CsA using RT-qPCR up to 10 days after infection. Experimentations were reproduced twice and with technical triplicates. Viral RNAs of SARS-CoV-2 Delta variant significantly decreased upon treatment with the highest concentration of CsA. No effect was observed in tissues infected with SARS-CoV-2 Omicron variant and HPIV3. Statistical significance between treated and nontreated conditions was calculated by determining the area under the curve and one-way ANOVA analysis (GraphPad Prism 10.0.1). Error bars represent mean with SEM. Cytokines expression (IL-8 and IFNλ respectively panels E and F) from HAE was determined by RNA detection of the cell lysates collected 4 DPI using RT-qPCR with SYBR Green. The housekeeping gene RNAse P was used for normalization (Thermo Fisher Scientific, ref. 4403326). Experimentations were performed as biological triplicates. The expression of IFNλ was significantly decreased in all HAE exposed to the highest concentration of CsA infected or not and at the low concentration in the noninfected HAE. The expression of IL-8 was significantly increased upon the highest concentration of CsA in infected HAE with SARS-CoV-2 but not with HPIV3. Statistical significance between treated and nontreated conditions was calculated using a one tailed t-test with unequal variance. ns: Nonsignificant, $^{*}p < 0.05$. Error bars represent mean with SEM. Expression is expressed as fold change relative to noninfected nontreated control (set as 1).

Omicron BA.1 variant.[5] Conversely, we found no evidence of CsA antiviral effect against SARS-CoV-2 Omicron variant or HPIV3 regardless of the dosage, suggesting a variant and a virus-specific effect. The research focused on CsA due to its routine use in post-transplant regimens. In vivo, it is challenging to distinguish the drug's direct antiviral effects from its immunosuppressive effects. The

ex vivo model of HAE provided a platform to evaluate CsA's impact on viral replication without confounding systemic immunosuppression effects.

This study also highlighted the complexity of CsA's antiviral effects, which may involve intricate inflammatory mechanisms and interactions with specific viral proteins. Our findings did not show an increase of viral replication under CsA and instead highlighted a diminished replication of SARS-CoV-2 delta variant at nontherapeutic concentrations. The mechanisms of CsA viral inhibition are thus complex and can vary depending on the virus.[6] A recent study showed that CsA exerts an antiviral effect against SARS-CoV-2 through Cyclophilin A (CypA).[11] CypA has been found to interact with the viral N protein and the nonstructural protein 1 of SARS-CoV-1. CypA also possesses its own PPi enzymatic activity, which appears to be important during CoV replication.[6] To the best of our knowledge, there are no data on the effect of CsA on HPIVs, and metapneumovirus. Previous studies found an interaction between CyPA and the N nucleocapsid protein of human RSV, a virus sharing some genetic similarities with HPIV3. Many explanations remain open to interpretation and deserve further investigations regarding the mechanism of action of CsA against each of these viruses.

CsA is known to have an anti-inflammatory effect by inhibiting the translocation of a family of transcription factors (NFAT), leading to reduced transcriptional activation of cytokine expression, like IL-2 and IL-6, and IFN-γ.[11] We therefore assessed the effect of CsA on the expression of two cytokines, IL-8 and IFNλ. At nontherapeutic concentration, we observed an opposite effect of CsA on IL-8 and IFNλ. In both uninfected and infected tissues, CsA reduced IFNλ expression, in line with its known anti-inflammatory effect, while in the same settings, CsA induced IL-8 expression.[12,13] Whether the latter observation is related to the ability of CsA to bind IL-8 as demonstrated by Bang and colleagues[11] deserves further investigations. Of note, at therapeutic concentrations, CsA did not affect viral replication nor IL-8 and IFNλ expression (except for a slight IFNλ reduction in noninfected tissues).

While this study provides valuable insights on the effects of CsA on respiratory viruses, particularly SARS-CoV-2, and on Il-8 and IFNλ expression, it has certain limitations. The changes in gene expression may not necessarily equate to changes in secreted cytokine. The model used did not assess the impact of CsA on T lymphocyte activation, and further studies are needed to understand the intricate dynamics of immunosuppressive treatments on respiratory viral infections comprehensively. Other models such as organ on chip (small microfluidic devices that allow human cells to perform complex organ-level functions in vitro) including immune cells might help to decipher the mechanisms.[14] In conclusion, this research suggests that at high concentrations, CsA may have a virus-specific antiviral effect, particularly against the SARS-CoV-2 Delta variant. Understanding the complexities of drugs' actions on different viruses is crucial for optimizing therapeutic approaches in transplant recipients and advancing our knowledge of immunosuppressive treatments' implications on respiratory viral infections.

AUTHOR CONTRIBUTIONS

LB and CT wrote the manuscript. SH, SC, SCL, MS, JLG, AB, LB, and CT revised it critically for important intellectual content. All authors approved the final version of the manuscript; moreover, all the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no relevant conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors (The data that support the findings of this study are available from the corresponding author upon reasonable request).

ETHICS STATEMENT

The project received the approval of the Swiss Ethic Committee. Project number 2023-00140 approved on March 7, 2023.

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