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BRIEF REPORT

Interferon-β-1a Inhibition of Severe Acute Respiratory Syndrome–Coronavirus 2 In Vitro When Administered After Virus Infection

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The ongoing coronavirus disease 2019 pandemic has forced the clinical and scientific community to try drug repurposing of existing antiviral agents as a quick option against severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2). Under this scenario, interferon (IFN) β-1a, whose antiviral potential is already known, and which is a drug currently used in the clinical management of multiple sclerosis, may represent as a potential candidate. In this report, we demonstrate that IFN-β-1a was highly effective in inhibiting in vitro SARS-CoV-2 replication at clinically achievable concentration when administered after virus infection.

Keywords. SARS-CoV-2; IFN-β-1a; COVID-19 clinical trial.

The current severe acute respiratory syndrome–coronavirus 2 (SARS-CoV-2) pandemic is severely affecting global health, putting an unprecedented strain on health facilities worldwide. The lack of effective direct-acting antiviral drugs and of immune modulatory therapies, validated through large population studies, worsens the scenario. While waiting for specific antivirals and vaccines to be developed, the biomedical community are also focused on drug repurposing: this is the case with hydroxychloroquine, viral protease inhibitors, and several immunomodulatory drugs already in clinical use for other indications [1].

In this scenario, interferons (IFNs) may also be considered, including IFN-β-1a, which has been widely used, and is still applied in some settings, for the management of relapsing-remitting multiple sclerosis [2]. At this time, several articles have already suggested that type I IFNs can interfere with coronavirus infections [3, 4]. In particular, the activity of IFN-β-1a has been described against SARS-CoV-1 both in vitro and in vivo, showing a protective effect on acute lung injury in a macaque model of infection [5, 6]. In the current study, we assessed its anti-SARS-CoV-2 activity in vitro to give a preclinical background to clinical trials evaluating the possible therapeutic role of IFN-β-1a in patients with coronavirus disease 2019 (COVID-19).

METHODS

Cells and Virus

Vero E6 cells (Vero C1008; clone E6–CRL-1586; American Type Culture Collection) were cultured in Dulbecco's modified Eagle medium supplemented with nonessential amino acids, penicillin/streptomycin, Hepes buffer, and 10% (vol/vol) fetal bovine serum (FBS). A clinical isolate of SARS-CoV-2 (hCoV-19/Italy/UniSR1/2020; GISAID accession no. EPI_ISL_413489) was obtained and propagated in Vero E6 cells.

Virus Titration

Virus stocks were titrated using both plaque reduction (plaque-forming units per milliliter) and end-point dilution (median tissue culture infective dose per milliliter) assays. In plaque reduction assays, confluent monolayers of Vero E6 cells were infected with eight 10-fold dilutions of virus stock. After 1 hour of adsorption at 37°C, the cell-free virus was removed. Cells were then incubated for 48 hours in Dulbecco's modified Eagle medium containing 2% FBS and 0.5% agarose. Cells were fixed and stained, and viral plaques were counted. In end-point dilution assays, Vero E6 cells (4×10^5 per well) were seeded into 96-well plates and infected with base 10 dilutions of virus stock. After 1 hour of adsorption at 37°C, the cell-free virus was removed, and complete medium was added to cells. After 48 hours, cells were observed to evaluate the cytopathic effect (CPE).

Infection Inhibition Experiment

Vero E6 cells were seeded into 96-well plates 24 hours before the experiment, and when at 95% confluency for each well, infected for 1 hour with SARS-CoV-2 at a multiplicity of infection (MOI) of 0.001 [7, 8]. Cells were washed with phosphate-buffered saline 1× to remove cell-free virus particles, and 200 μL of FBS-free medium containing different concentrations (5000 to 0.01 IU/mL) of IFN-β-1a (Avonex; Biogen Idec) was added to cells. The experiment ended 96 hours after infection. The possible drug toxicity of IFN-β-1a at a concentration of 5000 IU/mL was also tested on uninfected cells. Two experiments were performed in quadruplicate; live images were acquired (with an Olympus CKX41 inverted phase-contrast microscope) at 48, 72, and 96 hours after infection, and cell supernatants were collected for real-time quantitative

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reverse-transcription polymerase chain reaction (qRT-PCR) analysis at 48 and 72 hours after infection.

Viral RNA Extraction and qRT-PCR

The SARS-CoV-2 RNA relative amounts detected in each experimental condition as a cycle threshold (Ct) value were compared, with a mean Ct value determined for the positive infection control. The viral RNA was purified from 140 µL of all cell-free culture supernatant, using the QIAamp Viral RNA Mini Kit (Qiagen) and following the manufacturer's instructions. The purified RNA was subsequently used to perform the synthesis of first-strand complementary DNA, using the SuperScript First-Strand Synthesis System for RT-PCR (Thermo Fisher Scientific), following the manufacturer's instructions.

Real-time PCR, using the SYBR Green dye–based PCR amplification and detection method, was performed to detect the complementary DNA. We used the SYBR Green PCR Master Mix (Thermo Fisher Scientific), with the forward primer N2F (TTA CAA ACA TTG GCC GCA AA), the reverse primer N2R (GCG CGA CAT TCC GAA GAA), and the following PCR conditions: 95°C for 2 minutes, 45 cycles of 95°C for 20 seconds, annealing at 55°C for 20 seconds and elongation at 72°C for 30 seconds, followed by a final elongation at 72°C for 10 minutes. RT-PCR was performed using the ABI-PRISM 7900HT Fast Real Time instrument (Applied Biosystems) and optical-grade 96-well plates. Samples were run in duplicate, with a total volume of 20 µL.

Statistical Analysis

CPE cells observed were normalized to corresponding virus infection control and used to fit a curve with nonlinear regression for half-maximal effective concentration (EC_{50}) interpolation. The qRT-PCR results were analyzed, calculating the difference in Ct (ΔCt) as the difference between Ct values obtained for tested drug concentrations and for the infection control. Then 2-way analysis of variance and Dunnett multiple comparisons tests were performed to evaluate differences in ΔCt means evaluated for each group.

RESULTS

Antiviral Activity of IFN-β-1a

Vero E6 cells were treated with concentrations ranging from 5000 to 0.01 IU/mL of IFN-β-1a 1 hour after inoculation with SARS-CoV-2 and monitored for cytopathic effect and real-time-PCR quantitative evaluation at 48, 72, and 96 hours after infection. Inhibition of the SARS-CoV-2 by IFN-β-1a was dependent on both time and drug concentration. No morphological alterations related to drug toxicity was observed in uninfected cells treated with IFN-β-1a at 5000 IU/mL.

In particular, CPE was assessed at 48, 72, and 96 hours after infection (Figure 1A). First signs of CPE were already observed at the first time point, when cells were treated with low drug concentrations. Marked CPE was evident at 72 hours, showing that 10 IU/mL of the drug gave full protection from virus infection, while it was inhibited only partially with lower concentrations (5 to 0.1 IU/mL). As expected, 96-hour images showed that only higher concentrations of IFN-β-1a (5000 to 50 IU/mL) completely protected cells from SARS-CoV-2 infection. Lower tested concentrations (0.05 and 0.01 IU/mL) had no effect on hindering virus replication. Data were used for EC_{50} calculations at different time points, resulting in 1.971 IU/mL (95% confidence interval, .3969–4.891 IU/mL) at 48 hours, 2.071 IU/mL (.5982–5.819 IU/mL) at 72 hours, and 4.682 IU/mL (3.505–6.018 IU/mL) at 96 hours after infection (Figure 1B).

qRT-PCR Analysis

Cell supernatants collected 48 and 72 hours after infection from different cells treated with all drug concentrations were analyzed using RT-PCR. The results were fully comparable with CPE data (Figure 1C) for both time points, as Ct levels detected were inversely proportional to the amount of target nucleic acid in the sample. The ΔCt values were reported as the differences between Ct values for treated and untreated cells. Significant ΔCt values were observed down to the IFN-β-1a concentration of 5 IU/mL, at 48 hours ($P < .05$) and especially at 72 hours ($P < .001$). The Ct for 10 IU/mL was higher at 72 hours than at 48 hours (both $P < .001$), and results obtained with both 500 and 50 IU/mL concentrations were significantly different from the infection control at both time points ($P < .001$).

DISCUSSION

Several clinical trials on the administration of IFN to patients with COVID-19 are currently ongoing, even without experimental preclinical evidence of anti-SARS-CoV-2 potential [9] (https://www.hra.nhs.uk/covid-19-research/approved-covid-19-research/281317/). Among the IFNs currently available for clinical use, IFN-β-1a represents an interesting option, because its pharmacological features are well known. A very recent article, just released as a preprint during the submission of the current manuscript, describes the effect of IFN-β-1a when used before infection of cells with SARS-CoV-2 [10].

Our in vitro observations shed light for the first time on that antiviral activity of IFN-β-1a against SARS-CoV-2 when administered after the infection of cells, highlighting its possible efficacy in an early therapeutic setting. To this point, we detected that IFN-β-1a effectively inhibits both infectious virus particles and viral RNA on treated cells, when compared to virus-positive infection control without toxicity at its highest tested concentration. Moreover, the drug EC_{50} evaluated at 48, 72, and 96 hours after infection can be easily accessed in the clinical setting and could therefore help in addressing drug administration regimens in vivo [11]. From this perspective, it is important to note that, in our experimental setting, IFN-β-1a activity is retained up to 96 hours after its use on the infected cells.

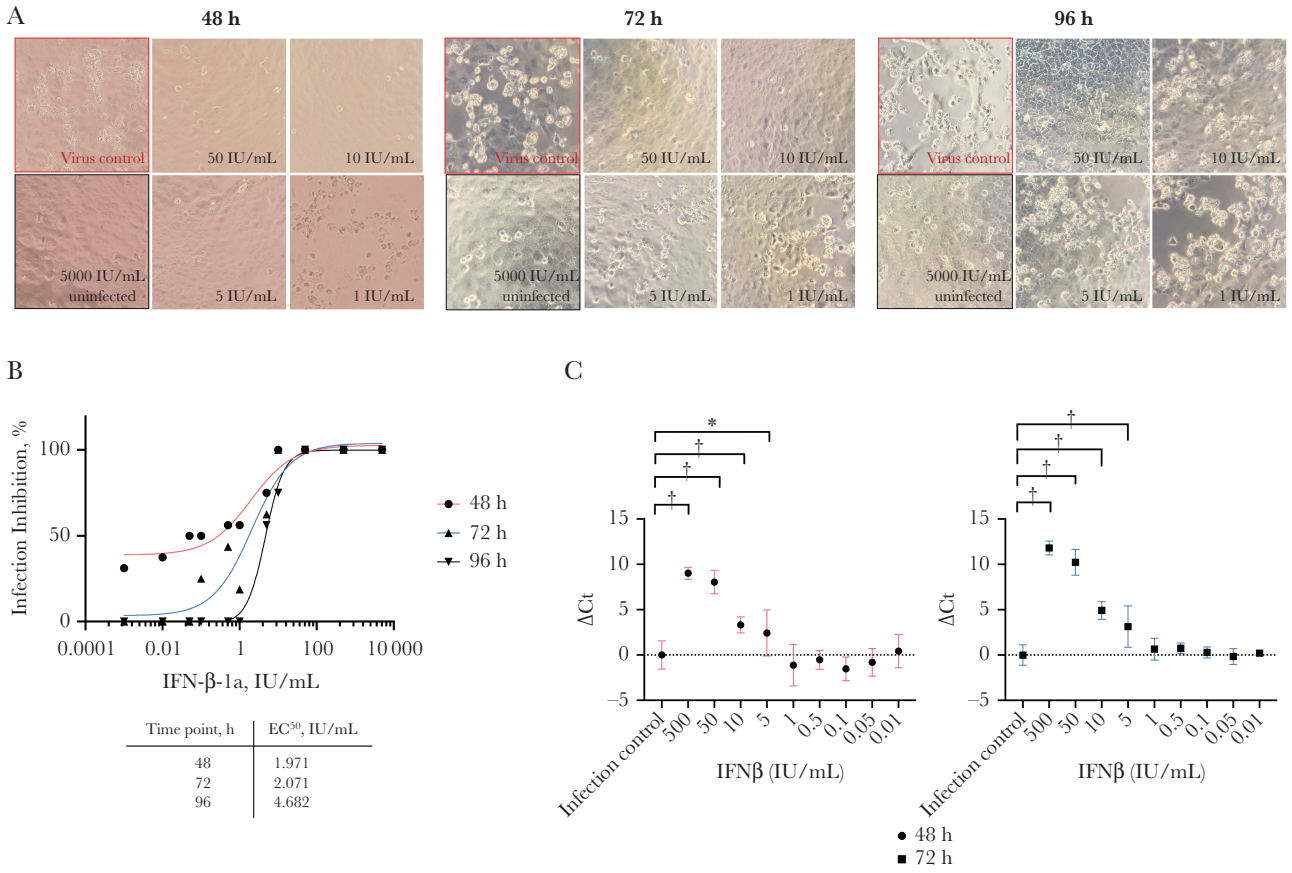

Figure 1. Antiviral activity of interferon (IFN) β-1a. *A,* Cytopathic effect (CPE) on infected cells treated with different concentrations of IFN-β-1a. Bright-field microscopic images (×20 magnification; 48, 72, and 96 hours after infection) show representative CPE of a clinical isolate of severe acute respiratory syndrome–coronavirus (hCoV-19/Italy/UniSR1/2020) detected on both untreated (virus control) and treated cells. Uninfected cells treated with 5000 IU/mL of IFN-β-1a are also shown. *B,* Half-maximal effective concentration calculated on CPE detected at 48, 72, and 96 hours after infection. *C,* quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis of cell supernatants at 48 and 72 hours after infection. Cycle threshold (Ct) levels are inversely proportional to the amount of target nucleic acid in the sample (the lower the Ct, the greater the amount of virus within the tested supernatant). The difference in Ct (ΔCt) is represented on the y-axes. Median values are shown for all experimental replicates, each tested in duplicate with qRT-PCR, and error bars represent 95% confidence intervals. *$P < .05$; †$P < .001$.

We are aware of the limitations of this preliminary study, such as the lack of a parallel evaluation of the activity IFN-β-1a on other viruses, such as vesicular stomatitis virus, whose clinical sensitivity to the drug is well known, to establish the level of susceptibility of SARS-CoV-2 to type I IFN. Moreover, owing to the lack of standardized phenotypic tests for this novel coronavirus, we have preferred to set the virus amount used for all assays on the CPE observed at the 3 time points (48, 72, and 96 hours after infection), rather than using a predetermined MOI. Hence, the antiviral activity of IFN-β-1a against SARS-CoV-2 was evaluated only at a single low MOI in a multiple-cycle replication condition, as previously reported for SARS-CoV-1 [7, 8].

It would also be interesting to test the activity of IFN-β-1a on other SARS-CoV-2 isolates featuring different phenotypic behaviors and possibly on animal models of COVID-19 to further assess, and dissect, the clinical potential of this therapeutic approach [12, 13]. This would have certainly have allowed a more complete evaluation of the clinical potential of IFN-β-1a activity in the clinical setting of COVID-19. Moreover, further in vitro testing on other cells of different IFNs, such as IFN-λ, may complement our preliminary results, it being of extreme importance to continue supporting IFN-based clinical trials [14].

Finally, we are fully aware that the preclinical evaluation of the antiviral activity of a drug, such as IFN-β-1a, is only a partial assessment of its possible clinical role in a disease such as COVID-19, in which the beneficial or detrimental effect of type I IFN is still to be established and in which immune-mediated damage is probably extremely important in determining the development of the worst outcomes of the infection [15]. Nonetheless, while we are surprised by the current lack of data on IFN-β-1a against SARS-CoV-2 in the literature, it is both urgent and clinically important to deliver data indicating whether type I may display direct antiviral activity against this virus. Obviously, its antiviral potential deserves further investigation in such an atypical setting.

Notes

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Author contributions. N.C. and N. M. conceived the study. R. F. and M. Castelli performed the reverse-transcription polymerase chain reaction–based experiments and analyzed data. N. C., E. C., and R. A .D. performed experiments and analyzed data. N. C., R. F., E. C., R. A. D., M. Castelli, C. S., G. A., M. Clementi, and N. M. analyzed all the results. All authors revised the manuscript, discussed the results, and contributed to the final manuscript.

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