Potent Antiviral Activities of Type I Interferons to SARS-CoV-2 Infection

Emily Mantlo[1], Natalya Bukreyeva[1], Junki Maruyama[1], Slobodan Paessler [1, 2] and Cheng Huang [1, 2] *

[1] Department of Pathology and [2] Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, Texas, United States of America

* Correspondence: chhuang@utmb.edu

Keywords: SARS-CoV-2; COVID-19; Interferon; antivirals; innate immune

Abstract:

The historical outbreak of COVID-19 disease not only constitutes a global public health crisis, but also has a devastating social and economic impact. The disease is caused by a newly identified coronavirus, Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2). There is an urgent need to identify antivirals to curtail the COVID-19 pandemic. Herein, we report the remarkable sensitivity of SARS-CoV-2 to recombinant human interferons α and β (IFNα/β). Treatment with IFN-α or IFN-β at a concentration of 50 international units (IU) per milliliter drastically reduce viral titers by 3.4 log or 4.5 log, respectively in Vero cells. The EC_{50} of IFN-α and IFN-β treatment is 1.35 IU/ml and 0.76 IU/ml, respectively, in Vero cells. These results suggested that SARS-CoV-2 is more sensitive to many other human pathogenic viruses, including the SARS-CoV. Overall, our results demonstrate the potent efficacy of human Type I IFN in suppressing SARS-CoV-2 replication, a finding which could inform future treatment options for COVID-19.

Introduction

The COVID-19 outbreak has started from the Wuhan Province, China since December 2019 and rapidly spread globally, causing over 752,000 confirmed cases and 36,000 deaths as of April 1, 2020. The causative agent for the COVID-19 disease is a newly identified Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) (1), which is transmitted through aerosol/airdrop inhalation or contact. The historical outbreak causes a public health crisis much severe than the SARS outbreak, which caused 8,098 infections and 774 deaths between November 2002 and July 2003. The COVID-19 disease also has a devastating social and economic impact worldwide. WHO has declared COVID-19 disease a pandemic. In USA, there is over 200,000 confirmed cases and 4,300 deaths as of April 1, 2020. It is estimated by CDC that the COVID-19 pandemic may claim 200,000 to 1.7 million lives in USA. Treatments are urgently needed. Due to the high rate of new infections reported each day, drugs already approved for clinical use for other disease may offer the most expedient option for treating COVID-19, and several such drugs are already being tested in clinical trials.

Type I interferons (IFN-α/β) are cytokines that play a pivotal role in inducing an antiviral response across a wide range of cell types. Humans produce 13 types of IFN-α and a singular IFN-β (2). Each Type I IFN ultimately induces a number of interferon-stimulated genes (ISGs) which encode for a variety of antiviral

effectors. Notably, IFN-β production leads to a positive feedback loop that further stimulates the expression of many of the IFN-α genes (3). Clinically, Type I IFNs have already approved for use in the treatment of certain cancers, autoimmune disorders, and viral infections. We assessed the sensitivity of SARS-CoV-2 to both IFN-α and IFN-β. Herein, we report that type I IFNs exhibited potent anti-SARS-CoV-2 activities in cultured cells, demonstrating the therapeutic potency of type I IFNs for COVID-19.

MATERIALS AND METHODS

Virus and Cells. The SARS-CoV-2 (USA-WA1/2020) were obtained from The World Reference Center for Emerging Viruses and Arboviruses (WRCEVA), University of Texas Medical Branch, Galveston, TX). Stock virus were propagated by infecting Vero cells (ATCC CCL-81) at a low multiplicity of infection (MOI) 0.0025. Three days after infection, supernatants were harvested and centrifuged at 2000 rpm for 5 min to remove cell debris. Stock virus was titrated with a 50% tissue culture infectious dose assay ($TCID_{50}$) (4). All experiments involving infectious virus were conducted at the University of Texas Medical Branch (Galveston, TX) in approved biosafety level 3 laboratories in accordance with institutional health and safety guidelines and federal regulations.

Virus growth curve. Vero cells were infected by SARS-CoV-2 at MOI 1 or 0.01 for 1 hr. Then inoculum was removed, replaced with media (DMEM+5%FBS) and incubated at 37^0C and 5% CO_2. At different time points after infection, supernatants were harvested and virus titers were determined by a $TCID_{50}$ assay on Vero cells.

Virus sensitivity to IFN treatment. Vero cells ($2x10^4$/well) were seeded into 48-well plates for 24 h and treated with human IFN-β1a (mammalian, cat# 11415, PBL) and IFN-α (Universal Type I alpha A/D (Bg III), PBL, cat# 11200-1) at different concentrations for 16 h. Cells were then infected with SARS-CoV-2 at an MOI of 0.01 $TCID_{50}$/cell. IFNs were supplemented after virus infection. Supernatants were collected at 22 hr post infection and assayed for virus titers.

Results

The growth kinetics of the newly identified SARS-CoV-2 in cultured cells remained to be characterized. Thus, we first examined the growth kinetics of SARS-CoV-2 in Vero cells. Vero cells were infected at either a low MOI (MOI=0.01) or high MOI (MOI=1). Supernatant was collected every 8-16 hours. At both conditions, viral

titers peaked at around 24 hours post-infection (hpi) and remained stable until 40 hours post-infection before declining (Fig. 1). The peak virus titer was $5.5x10^6$ $TCID_{50}$/ml at MOI 0.01 and $3.75x10^5$ $TCID_{50}$/ml at MOI, indicating that viral replication was more efficient at low MOI (MOI=0.01) than high MOI (MOI=1). Virus infection also caused strong cytotoxic effect (CPE), which was more evident at 48 hpi and later than the peak of virus production (at 40 hpi).

Next, we examined the effect of recombinant human IFN-α and IFN-β treatment on viral infection. Vero cells were pre-treated with different concentrations of IFN-α or IFN-β ranging from 50-1000 international units (IU) per milliliter for 16 hours prior to infection. After 1 hour of infection with SARS-CoV-2 (MOI 0.01), media containing IFN was returned, and cells were incubated for a further 22 hours. Supernatants were then collected, and viral titers were determined via $TCID_{50}$ assay. The result indicated a potent inhibition of SARS-CoV-2 infection by IFN-α treatment. Virus titers were not detectable except at the lowest concentration tested (50 IU/ml), at which IFN-α drastically reduced viral titers by 4-logs of magnitude (Fig. 2). IFN-β exhibited more potent anti-SARS-CoV-2 activity than IFN-α: the virus titer was below the detection limit at all concentrations tested (50 u/ml-1000u/ml). Consistently, no CPE was observable under microscope examination in all IFN-treated samples.

We next tested the antiviral efficacy of IFN-α and IFN-β at lower concentrations ranging from 1-50 IU/ml. For both IFNs, a dose-dependent effect was clearly observed using these lower concentrations (Fig. 3). For IFN-α, the anti-SARS-CoV-2 activity could be noticed as low as 5 IU/ml, at which the virus titer was significantly reduced by over 1 log ($P<0.01$). With increasing IFN-α concentrations, the virus titers steadily decreased. For IFN-β, treatment with 1 IU/ml of IFN-β resulted in a moderate (approximately 70%) but significant decrease in virus titer ($P<0.05$, Student t test). Virus levels were nearly undetectable upon treatment with 10, 25, and 50 IU/ml of IFN-β. The EC_{50} of IFN-α and IFN-β treatment is 1.35 IU/ml and 0.76 IU/ml, respectively. Taken together, these results indicated that treatment with low concentrations of both IFN-α and IFN-β significantly inhibited viral replication, with IFN-β being slightly more effective than IFN-α.

Discussion

Our data clearly demonstrated that SARS-CoV-2 is highly sensitive to both IFN-α and IFN-β treatment in cultured cells. The experiment was performed in the IFN-α/β gene-defective Vero cells. It is plausible that the anti-SARS-CoV-2 efficacy of exogenous IFN treatment is more potent in IFN-competent cells, as IFN-β is known to mediate the expression of other subtypes of Type I IFNs. Our data may also explain, at least in part, that approximately 80% of patients actually only developed mild symptoms and recovered (5). It is possible that many infected people developed IFN-α and IFN-β-mediated innate immune response upon SARS-CoV-2 infection, which limits virus infection during initial stage. Later on, adaptive immune response, such as antibody production, may eventually help the recovery from the COVID-19 disease.

Compared to SARS-CoV-2, it seems SARS-CoV is not relatively less sensitive to IFN treatment *in vitro* (6, 7). One study reported that the EC_{50} of IFN-β for SARS-CoV is 95 or 105 IU/ml depending on virus strain (8). Many other highly pathogenic viruses are also resistant to exogenous IFN treatment. Treatment of Ebola-infected cells with exogenous IFN-α does not lead to any reduction in viral replication (9), which is hypothesized to be a result of viral protein antagonism of the IFN response. Also, arenavirus Junín virus, which causes Argentine Hemorrhagic Fever, is also found to be insensitive to IFN treatment. In Vero cells, the titers of JUNV were reduced by less than 1-log when treated with a high concentration of human IFN-α, β or γ (1000 U/ml) (10). By contrast, the remarkable sensitivity of SARS-CoV-2 to IFN treatment reveals a weakness of this virus, which may be informative to antiviral development. Further work is needed to characterize the IFN response during SARS-CoV-2 infection and any possible viral interference.

In vitro, we have demonstrated that SARS-CoV-2 replication is inhibited by clinically-achievable doses of both IFN-α and IFN-β. Thus, the use of IFN may be a viable treatment option for COVID-19. Serum levels of Roferon-A and Intron-A, two recombinant IFN-α drugs already approved for the treatment of hepatitis B and C, can reach up to 330 IU/ml and 204 IU/ml, respectively (11). Betaferon and Rebif, two recombinant IFN-β drugs approved for use in the treatment of multiple sclerosis, can reach respective concentrations of 40 IU/ml and 4.1 IU/ml in the serum (11). Some of these drugs may therefore have the potential to be repurposed for the treatment of COVID-19 either alone or in combination with other antiviral therapies.

Acknowledgments

We thank Drs. Kenneth Plante (The World Reference Center for Emerging Viruses and Arboviruses, UTMB) and Natalie Thornburg from the CDC for providing the SARS-CoV-2 stock virus. Work in Dr. Paessler laboratory was supported in parts by Public Health Service grants RO1AI093445 and RO1AI129198 and the John. S. Dunn Distinguished Chair in Biodefense endowment. C.H. was supported by UTMB Commitment Fund P84373 and would like to acknowledge the Galveston National Laboratory (supported by the Public Health Service award 5UC7AI094660) for support of his research activity.

Figure legend:

Figure 1: Vero cells were infected by SARS-CoV-2 at MOI 1 or 0.01 for 1 hr. At different time points after infection, supernatants were harvested and virus titers were determined by a $TCID_{50}$ assay on Vero cells. The average of triplicates and Standard deviation are shown. Dotted line indicates the detection limit.

Figure 2: Vero cells were pretreated with human IFN-α or IFN-β (0, 50, 125, 250, 500, 1000 IU/ml) for 16 hours. Cells were then infected with SARS-CoV2 for 1 hour at an MOI of 0.01. Viral inoculums were removed and replaced with fresh media containing listed concentrations of IFN-α or IFN-β. Media was collected at 22 hpi and titers were determined via TCID50 assay on Vero cells. The average of triplicates and Standard deviation are shown. Dotted line indicates the detection limit.

Figure 3: Vero cells were pretreated with human IFN-α or IFN-β (0, 1, 5 , 10, 25 , 50 U/ml) for 16 hours and then infected with SARS-CoV2 at an MOI of 0.01. Viral inoculums were removed and replaced with fresh media containing listed concentrations of IFN-α or IFN-β. Media was collected at 22 hpi and virus titers were determined via TCID50 assay. The average of triplicates and Standard deviation are shown. Dotted line indicates the detection limit. (*, $P<0.05$; **, $P<0.01$; n.s. not significant, one tail Student T test)

References

1. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270-3. Epub 2020/02/06. doi: 10.1038/s41586-020-2012-7. PubMed PMID: 32015507; PMCID: PMC7095418.

2. Pestka S, Krause CD, Walter MR. Interferons, interferon-like cytokines, and their receptors. Immunol Rev. 2004;202:8-32. doi: 10.1111/j.0105-2896.2004.00204.x. PubMed PMID: 15546383.

3. Honda K, Takaoka A, Taniguchi T. Type I interferon [corrected] gene induction by the interferon regulatory factor family of transcription factors. Immunity. 2006;25(3):349-60. doi: 10.1016/j.immuni.2006.08.009. PubMed PMID: 16979567.

4. Narayanan K, Huang C, Lokugamage K, Kamitani W, Ikegami T, Tseng CT, Makino S. Severe acute respiratory syndrome coronavirus nsp1 suppresses host gene expression, including that of type I interferon, in infected cells. Journal of virology. 2008;82(9):4471-9. Epub 2008/02/29. doi: 10.1128/JVI.02472-07. PubMed PMID: 18305050; PMCID: PMC2293030.

5. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 2020. Epub 2020/02/25. doi: 10.1001/jama.2020.2648. PubMed PMID: 32091533.

6. Moriguchi H, Sato C. Treatment of SARS with human interferons. Lancet. 2003;362(9390):1159. doi: 10.1016/S0140-6736(03)14484-4. PubMed PMID: 14550718.

7. Dahl H, Linde A, Strannegård O. In vitro inhibition of SARS virus replication by human interferons. Scand J Infect Dis. 2004;36(11-12):829-31. doi: 10.1080/00365540410021144. PubMed PMID: 15764169.

8. Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. Lancet. 2003;361(9374):2045-6. Epub 2003/06/20. doi: 10.1016/s0140-6736(03)13615-x. PubMed PMID: 12814717.

9. Kash JC, Mühlberger E, Carter V, Grosch M, Perwitasari O, Proll SC, Thomas MJ, Weber F, Klenk HD, Katze MG. Global suppression of the host antiviral response by Ebola- and Marburgviruses: increased antagonism of the type I interferon response is associated with enhanced virulence. J Virol. 2006;80(6):3009-20. doi: 10.1128/JVI.80.6.3009-3020.2006. PubMed PMID: 16501110; PMCID: PMC1395418.

10. Huang C, Walker AG, Grant AM, Kolokoltsova OA, Yun NE, Seregin AV, Paessler S. Potent inhibition of Junin virus infection by interferon in murine cells. PLoS neglected tropical diseases. 2014;8(6):e2933. Epub 2014/06/06. doi: 10.1371/journal.pntd.0002933. PubMed PMID: 24901990; PMCID: PMC4046933.

11. Strayer DR, Dickey R, Carter WA. Sensitivity of SARS/MERS CoV to interferons and other drugs based on achievable serum concentrations in humans. Infect Disord Drug Targets. 2014;14(1):37-43. doi: 10.2174/1871526514666140713152858. PubMed PMID: 25019238.