Short communication

Inactivation of SARS-CoV-2 isolates from lineages B.1.1.7 (Alpha), P.1 (Gamma) and B.1.110 by heating and UV irradiation

S. Ulloa [a], C. Bravo [a], E. Ramirez [b], R. Fasce [b], J. Fernandez [a,*]

[a] *Molecular Genetics Sub Department, Institute of Public Health of Chile, Santiago, Chile*
[b] *Viral Diseases Sub Department, Institute of Public Health of Chile, Santiago, Chile*

ARTICLE INFO

ABSTRACT

Currently, the rapid global spread of SARS-CoV-2 is related to G clade (including GH, GR, GRY and GV clades), which are associated with more than 98 % of sequenced viral isolates worldwide. The unprecedented velocity of spread of SARS-CoV-2 outbreak represents a critical need for prevention strategies. Vaccines are recently being available and antiviral drugs have shown limited efficacy in COVID-19 patients. Thus, it is needed to know how to reduce the infectivity of the virus by different physicochemical conditions in order to prevent exposure to contaminated material.

This work describes heating and irradiating UV-C light procedures to reduce the infectivity of SARS-CoV-2 belonging to different three lineages. Results of physicochemical treatment showed no differences among viral lineages. Analytical conditions for efficient inactivation of SARS-CoV-2 were determined.

1. Introduction

The current COVID pandemic has generated 176,380,186 infected people and 3,814,228 deaths worldwide until June 15 (https://coronavirus.jhu.edu/map.html). The disease-causing virus was identified as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) due to its similarity to SARS-CoV (Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020). Eight major clades are defined by GISAID, based on marker mutations, grouping SARS-CoV-2 in. S, L, V, G, GH, GR, GRY and GV (https://www.gisaid.org/). These clades are subdivided into major lineages from A to Z and subsequently in lineages (for example A.2 and A.2.1) (Rambaut et al., 2021).

In february 2021, the World Health Organization (WHO) defined the "SARS-CoV-2 variant of concern" (VOC) and "SARS-CoV-2 variant of interest" (VOI) as viral isolates from lineages with highest public health implications (World Health Organization, 2021). By the middle of June 2021, a WHO label was added to these variants as follow for VOCs: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and for VOIs: Epsilon (B.1.427/429), Zeta (P.2), Eta (B.1.525), Theta (P.3), Iota (B.1.526), Kappa (B.1.617.1) and Lambda (C.37). By June 15, almost 25 % of the total sequences submitted to GISAID belonged to Alpha variant; 5% to Gamma variant and 70 % to Delta and Eta variants. From the total

(3495) of Chilean sequences submitted by that date 22.6 % corresponded to Gamma, 19.2 % to Lambda, 3.75 % to Alpha, 0.6 % to Epsilon, 0.5 % to Zeta, 0.4 % to Iota and 0.05 % to Beta. Moreover, B.1.110 lineage was present in 3.5 % of this total, and is characterized in cov-lineages.org as a Chilean, American and Australian lineage (O'Toole et al., 2021).

The G, GR (P.1 included), GRY (B.1.1.7 included), GH (B.1.110 included) and GV clades are associated with more than 98 % of sequenced circulating viruses (https://www.gisaid.org/). All they have a spike protein variant, and pseudotyped viruses expressing Gly614 instead Asp614 produces a virus with higher infectivity than wild type variant, which has been correlated with increased thermostability (Long et al., 2020). Despite this, heat, detergents and ultraviolet (UV) irradiation have been described as useful for inactivating SARS-CoV-2 samples and/or decontaminating work areas and sample processing elements (Heilingloh et al., 2020; Inagaki et al., 2020; Patterson et al., 2020; Biasin et al., 2021). However, these studies do not show the response of SARS-CoV-2 isolates from different lineages to different temperature or UV irradiation conditions.

In this work, we showed how three isolates belonging to lineages P.1 (VOC), B.1.1.7 (VOC) and B.1.110 were affected by heat using different temperatures and UV irradiation at different dosages.

2. Material and methods

2.1. SARS-CoV-2 isolation

Three clinical isolates of SARS-CoV-2, previously confirmed as positive by rRT-PCR and classified in lineages B.1.110, P.1 and B.1.1.7 by whole-genome sequencing, were isolated from nasopharyngeal swabs collected in tubes containing UTM-RT mini transport media (Copan Diagnostics Inc.) in a BSL3 laboratory. Monolayers of Vero E6 cells (ATCC® CRL-1586™) were seeded in MEM containing 10 % (v/v) fetal calf serum and cultured at 37 °C in a 5% carbon dioxide atmosphere. After seven days of incubation, all tissue cultures showed SARS-Cov-2 cytopathic effect. Virus stocks were collected by centrifuging supernatants of infected cells at 3000 rpm for 10 min and stored at −80 °C. Viral titers were determined by endpoint dilution assay: serial dilutions of virus samples were incubated at 37 °C for 4 days and subsequently examined for cytopathic effect (CPE) in infected cells, calculating the 50 % tissue culture infective dose ($TCID_{50}$).

2.2. Inactivation of SARS-CoV-2 by temperature

To determine the susceptibility of SARS-CoV-2 to temperature, 200 u L aliquots of virus normalized at $10^{8.5}$ $TCID_{50}$/mL in Phosphate-Buffered Saline (PBS) were incubated in triplicates in 1.5 mL polypropylene tubes using a heating block (Thermomixer comfort, Eppendorf) to set four different temperatures: 45, 50, 55 and 60 °C in different times up to 60 min. After exposure, the $TCID_{50}$/mL of each sample was determined by endpoint dilution using seven serial ten-fold dilutions, as described before. An unheated virus suspension was used as a negative control.

2.3. Inactivation of SARS-CoV-2 by UV-irradiation

To determine the susceptibility of SARS-CoV-2 to UV-C irradiation, 200 u L aliquots of virus normalized at $10^{8.5}$ $TCID_{50}$/mL in PBS were irradiated in triplicates with UV-C light for up to 30 min in the six upper wells of flat-bottomed 24-well plates. Those upper wells were irradiated at a distance of 60 cm with one lamp UV-C light source in a Class II type A2 laminar flow biosafety cabinet (NuAire). Irradiation with 0.1 mW/

cm^2 was in times 2.5, 5, 7.5, 10, 12.5, 15, 20 and 30 min and, each dose corresponding to 15, 30, 45, 60, 75, 90, 120 and 180 mJ/cm^2, respectively. After exposure, the $TCID_{50}$/mL of each sample was determined by endpoint dilution using seven ten-fold dilutions, as described before. An unirradiated virus suspension was used as a negative control.

3. Results

When the isolates of lineages B.1.110, P.1 and B.1.1.7 of SARS-CoV-2 were incubated at different temperatures, they showed to be susceptible to heat: at 45 °C the log10 of $TCID_{50}$/mL after 60 min decreased from 8.5 to 2.5, 2.5 and 1.5 in each isolate, respectively. At 50 °C, the log10 of $TCID_{50}$/mL after 30 min decreased from 8.5 to 2, 0.5, and 2 in each isolate, respectively. At 55 °C, the log10 of $TCID_{50}$/mL after 10 min decreased from 8.5 to 1.5, 1.5 and 2 in each isolate, respectively. At 60 °C, the log10 of $TCID_{50}$/mL decreased from 8.5 to 0.5 for the three isolates before 10 min of incubation (Fig. 1).

The three isolates also showed high susceptibility to UV-C irradiation, decreasing from 8.5 to 0.5 log10 $TCID_{50}$/mL after 30 min of exposition into the biosafety cabinet (Fig. 2).

4. Discussion

The current global SARS-CoV-2 pandemic is of urgent concern due its high transmission rate and spread throughout the world. Although currently there are vaccines available, however, the high case number at world level (incidence) is related to vaccine escape (Thompson et al., 2021) and there are reports about some escape variants (Ikegame et al., 2021; Collier et al., 2021; Zhou et al., 2021). Thus, there is a fundamental need to know how to reduce the virus infectivity and inactivate it under different physicochemical conditions, in order to reduce the risk of contagion by contaminated surfaces in laboratories working with COVID samples or healthcare facilities.

The $TCID_{50}$ assay is an endpoint dilution assay that determines the point at which 50 % of cells in a culture are infected (Ramakrishnan, 2016), relating those results with the viral infectivity or viral inactivation. Heat was the first way for viral inactivation: viral isolates from the three lineages were incubated at several temperatures showing different results. At 45 °C, $TCID_{50}$/mL decreased by half after 10 min, but after
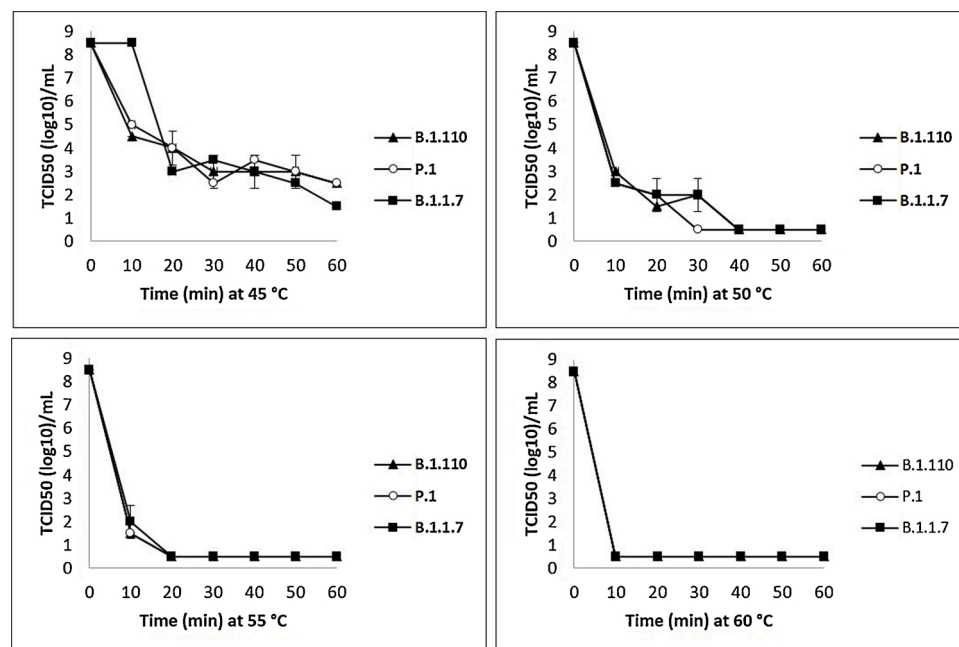

Fig. 1. Effect of heating on the infectivity of SARS-CoV-2. Virus aliquots (200 μL at $10^{8.5}$ $TCID_{50}$/mL and ten-fold dilutions, in triplicates) of 3 isolates from lineages B.1.110, P.1 and B.1.1.7 were incubated at 45, 50, 55 and 60 °C. Samples were removed at the designated time and titrated in Vero E6 cells.
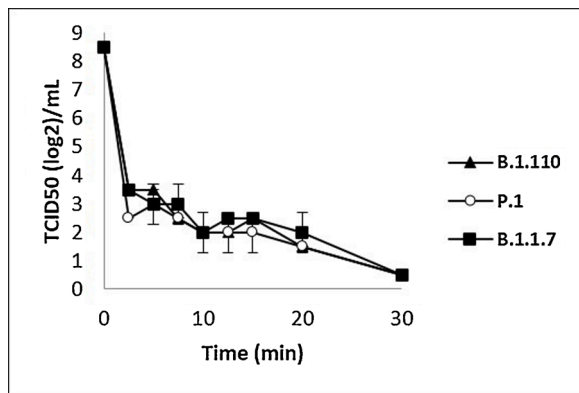
Fig. 2. Effect of UV-C irradiation on the infectivity of SARS-CoV-2 into a laboratory biosafety cabinet. Virus aliquots (200 μL at $10^{8.5}$ TCID$_{50}$/mL and tenfold dilutions, in triplicates) of 3 isolates from lineages B.1.110, P.1 and B.1.1.7 were irradiated using an UV lamp at 60 cm distance above the bottom of 24-well plates. Samples were removed at the designated time and titrated in Vero E6 cells.

60 min virus was still infective, indicating that this temperature was not enough to inactivate the virus. In spite of this, B.1.110 and P.1 isolates showed to be more resistant than B.1.1.7 isolate at 45 °C after 60 min of treatment. Heated viruses at 50 °C showed differences after 30 min, being P.1 isolate more sensitive to temperature. The three isolates were completely inactivated after 40 min at 50 °C, probably achieving the lower limit of detection of this assay (0.5 TCID$_{50}$/mL), because subsequent treatments with longer time of incubation did not decrease the infectivity, even using higher temperatures. At 55 °C, SARS-CoV-2 was inactivated after 20 min and after 10 min at 60 °C.

These results are slightly different to previously described for SARS-CoV-1, in which inactivation was achieved at 65 °C for 20 min (Darnell et al., 2004; Inagaki et al., 2020) and 67 °C for 60 min (Duan et al., 2003) but similar to other study that indicates SARS-CoV-2 could be inactivated heating it at 56 °C for 30 min, 70 °C for 10 min or 90 °C for 5 min (Xiling et al., 2021).

When the three isolates were irradiated using UV-C light into the biosafety cabinet, B.1.110 and B.1.1.7 isolates reduced their infectivity first than P.1 at 2.5 min of exposition. However, after 30 min the three isolates were completely inactivated, indicating the three lineages could be equally sensitive to UV irradiation. It is important to highlight that in this study the virus was inactivated irradiating UV light at work distance 60 cm (a standard distance in a biosafety cabinet). This protocol showed similar results to other studies performed with very short distance (2−3 cm) for irradiation treatments (Darnell et al., 2004; Heilingloh et al., 2020; Inagaki et al., 2020) and the UV dosages to achieve viral inactivation were 112.5 and 1048 mJ/cm2, respectively (Inagaki et al., 2020; Heilingloh et al., 2020). In this work, the UV dosage necessary to inactivate the virus was approximately 180 mJ/cm2 using both a biosafety cabinet (1 lamp), similar to Inagaki et al.. This finding can be an important additional measure in preventing the spread of SARS-CoV-2 infection in laboratory practices because most microbiology laboratories have a biosafety cabinet, and these results demonstrated this UV dosage was useful to inactivate SARS-CoV-2 in high infection titer samples. Consequently, heat inactivation and UV-C light inactivation are strongly recommended as chemical-free methods for applying in laboratories or healthcare facilities.

The G clade was described before as more resistant to heat than isolates from L and S clades (Long et al., 2020), but the sensitivity to UV-C irradiation in different clades or lineages has not been studied yet. In this work, three G clade-isolates, considering their globally spread and predominance, were selected: B.1.1.7 (GRY, Alpha), P.1 (GR, Gamma) and B.1.110 (GH). Two of them (Alpha and Gamma) were designated as VOC by WHO and the third is a not relevant lineage in

terms of public health but it is present in more than 3% of sequenced Chilean isolates. The three isolates were tried and no significant differences were found among them, thus there would be no relationship between a higher resistance to heat or UV-C irradiation and the type of variant studied. Additional studies including more isolates from different lineages and clades are necessary to corroborate these suggestions.

In conclusion, SARS-CoV-2 is susceptible to temperature and UV-C light: a short time of incubation (10 min) at 60 °C or UV-C irradiation for 30 min of exposition in a biosafety cabinet (1 lamp) were effective to inhibit the viral infectivity in high infection titer samples. However, lower temperatures could be also recommended at different times: 40 min at 50 °C or 20 min at 55 °C. These procedures could be useful to decontaminate work areas and personal protective equipment used in biological sample processing to prevent laboratory-acquired infections or in healthcare facilities. Also, understanding different ways for SARS-CoV-2 inactivation will allow an easier and safer manipulation of the virus in laboratories with fewer resources.

Ethical statement

Given the important impact that data from this study could have on Public Health, all nasopharyngeal samples from COVID-19 positive patients were unidentified and not considered as Human samples.

Author's contribution

Ulloa S and **Bravo C**: conceptualization, methodology, validation, formal analysis, writing and visualization. **Ramirez E**: methodology and critical review. **Fasce R**: resources. **Fernandez J**: methodology, resources and supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

References

Biasin, Mara, Bianco, Andrea, Pareschi, Giovanni, Cavalleri, Adalberto, Cavatorta, Claudia, Fenizia, Claudio, Galli, Paola, et al., 2021. UV-C irradiation is highly effective in inactivating SARS-CoV-2 replication. Sci. Rep. 11 (1), 6260.

Collier, Dami A., De Marco, Anna, Ferreira, Isabella A.T., Bo, Meng, Datir, Rawlings P., Walls, Alexandra C., Kemp, Steven A., et al., 2021. Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies. Nature 593 (7857), 136–141.

Coronaviridae Study Group of the International Committee on Taxonomy of Viruses, 2020. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat. Microbiol. 5 (4), 536–544.

Darnell, Miriam E.R., Subbarao, Kanta, Feinstone, Stephen M., Taylor, Deborah R., 2004. Inactivation of the coronavirus that induces severe acute respiratory syndrome, SARS-CoV. J. Virol. Methods 121 (1), 85–91.

Duan, Shu-Ming, Zhao, Xin-Sheng, Wen, Rui-Fu, Huang, Jing-Jing, Pi, Guo-Hua, Zhang, Su-Xiang, Han, Jun, et al., 2003. Stability of SARS coronavirus in human specimens and environment and its sensitivity to heating and UV irradiation. Biomed. Environ. Sci. 16 (3), 246–255.

Heilingloh, Christiane Silke, Aufderhorst, Ulrich Wilhelm, Schipper, Leonie, Dittmer, Ulf, Witzke, Oliver, Yang, Dongliang, Zheng, Xin, et al., 2020. Susceptibility of SARS-CoV-2 to UV irradiation. Am. J. Infect. Control 48 (10), 1273–1275.

Ikegame, Satoshi, Siddiquey, Mohammed, Hung, Chuan-Tien, Haas, Griffin, Brambilla, Luca, Oguntuyo, Kasopefoluwa, Kowdle, Sheryas, et al., 2021. Neutralizing Activity of Sputnik V Vaccine Sera Against SARS-CoV-2 Variants. Research Square. https://doi.org/10.21203/rs.3.rs-400230/v1. April.

Inagaki, Hiroko, Saito, Akatsuki, Sugiyama, Hironobu, Okabayashi, Tamaki, Fujimoto, Shouichi, 2020. Rapid inactivation of SARS-CoV-2 with deep-UV LED irradiation. Emerg. Microbes Infect. 9 (1), 1744–1747.

Long, S..Wesley, Wesley Long, S., Olsen, Randall J., Christensen, Paul A., Bernard, David W., Davis, James J., Shukla, Maulik, et al., 2020. Molecular architecture of early dissemination and massive second wave of the SARS-CoV-2 virus in a major metropolitan area. mBio 11 (6). https://doi.org/10.1128/mBio.02707-20.

O'Toole, Áine, Hill, Verity, Pybus, Oliver G., Watts, Alexander, Bogoch, Issac I., Khan, Kamran, Messina, Jane P., et al., 2021. Tracking the international spread of SARS-CoV-2 lineages B.1.1.7 and B.1.351/501Y-V2. Wellcome Open Res. 6 (May), 121.

Patterson, Edward I., Prince, Tessa, Anderson, Enyia R., Casas-Sanchez, Aitor, Smith, Shirley L., Cansado-Utrilla, Cintia, Solomon, Tom, et al., 2020. Methods of inactivation of SARS-CoV-2 for downstream biological assays. J. Infect. Dis. 222 (9), 1462–1467.

Ramakrishnan, Muthannan Andavar, 2016. Determination of 50% endpoint titer using a simple formula. World J. Virol. 5 (2), 85–86.

Rambaut, Andrew, Holmes, Edward C., O'Toole, Áine, Hill, Verity, McCrone, John T., Ruis, Christopher, du Plessis, Louis, Pybus, Oliver G., 2021. Addendum: a dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. Nat. Microbiol.

Thompson, Robin N., Hill, EdwardM., Julia, R.Gog., 2021. SARS-CoV-2 incidence and vaccine escape. Lancet Infect. Dis. https://doi.org/10.1016/S1473-3099(21)00202-4. April.

World Health Organization, 2021. COVID-19 Weekly Epidemiological Update. June 2021. https://apps.who.int/iris/bitstream/handle/10665/341716/CoV-weekly-sit rep8Jun21-eng.pdf.pdf?sequence=1.

Xiling, Guo, Yin, Chen, Ling, Wang, Xiaosong, Wu, Jingjing, Fan, Fang, Li, Xiaoyan, Zeng, et al., 2021. In vitro inactivation of SARS-CoV-2 by commonly used disinfection products and methods. Sci. Rep. 11 (1), 2418.

Zhou, Daming, Dejnirattisai, Wanwisa, Supasa, Piyada, Liu, Chang, Mentzer, Alexander J., Ginn, Helen M., Zhao, Yuguang, et al., 2021. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. Cell 184 (9), 2348–2361 e6.