Simulated Sunlight Decreases the Viability of SARS-CoV-2 in Mucus

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Abstract

This study examined the effect of simulated sunlight on the viability of SARS-CoV-2 spiked into tissue culture medium or mucus. The study revealed that inactivation took 37 minutes in medium and 107 minutes in mucus. These times-to-inactivation were unexpected since they are longer than have been observed in other studies.

Main Text

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been responsible for a global pandemic of its associated disease, COVID-19, since its emergence in Wuhan, China in December of 2019 (1). The ability of the virus to remain infectious on non-porous surfaces has been estimated to range from 3 to 28 days depending on ambient conditions (2,3,4), which has a potential role in transmission through fomites, particularly on high-touch surfaces. Recent studies have examined the ability of sunlight to inactivate SARS-CoV-2 in simulated saliva, with intriguing results (5). Here, we investigate the ability of sunlight to act as a natural sterilizing medium and reduce the viability of mucus-suspended SARS-CoV-2.

Detailed methods are described in the Appendix. SARS-CoV-2 was diluted in either tissue culture medium or a mucus-simulating organic matrix and allowed to dry on stainless steel coupons. Coupons were then set away from (control coupons) or under (experimental coupons) the light source of a SunLite Solar Simulator Model 11002 (Abet Technologies) set to 100 mV (i.e. 1.28 W/m^2 UVB), approximately equivalent to the irradiance measured at noon on the 2020 spring and fall equinoxes (March 21 and September 22) at 40°N latitude and sea level (National Center for Atmospheric Research Tropospheric Ultraviolet and Visible Radiation Model). In experiments controlling for heat, coupon temperature, measured using a digital thermometer and thermocouple, was kept at room temperature (22.5°C) using a cooling block. Relative humidity was measured with a hygrometer. At selected time-points post-desiccation, the virus was re-suspended in medium and used to inoculate Vero cells in a TCID₅₀ assay. Cytopathic effect was observed 4 days later and log₁₀ TCID₅₀ values plotted to compare virus viability. Linear regression analysis, performed using GraphPad Prism, was used to evaluate statistical significance between outcomes.

The figure demonstrates that the viability of SARS-CoV-2 decreased significantly faster when exposed to sunlight versus exposure to the same conditions with sunlight removed ($p \leq 0.0045$). Viral titer decreased most rapidly when the virus was suspended in culture medium, from 3.12 to 1.5 log₁₀ TCID₅₀/mL in 37 minutes at 22.5°C and 34% RH. Although not statistically significant, the same reduction in viability took 47 minutes during heat- and RH-variable assays ($p = 0.4014$). The presence of an organic matrix significantly extended the survival of the virus when exposed to sunlight ($p = 0.0096$ and 0.0036 for heat-controlled and heat-variable assays, respectively). In mucus, sunlight exposure for 147 minutes was necessary to reduce virus titer from 3.12 to 1.5 log₁₀ TCID₅₀/mL at room temperature. This was reduced to 107 minutes when heat and RH were variable, but the difference was not statistically significant ($p = 0.3361$).

The present study demonstrates that 1.28 W/m² of UVB radiation is capable of inactivating SARS-CoV-2 desiccated on stainless steel surfaces. The matrix within which the virus is suspended has a demonstrable impact on the effect of sunlight as a disinfection agent. Inactivation of a 3.12 log inoculum occurred faster when simple media was used (37-47 minutes) compared to simulated mucus (107-147 minutes). This study did not demonstrate a significant difference in inactivation time based on changes in temperature and RH, which stands in contrast to previous published data (6). This may be due to the relatively small variation in ambient temperature and RH between the experimental conditions. Putting these results in context, we observed a much slower inactivation time compared to a recent report by Ratnesar-Shumate et al. (5), who demonstrated medium-suspended SARS-CoV-2 being inactivated at a rate almost twice that seen in our investigation. Moreover, when the virus was spiked into simulated saliva in the aforementioned study, the rate of viral inactivation was almost nine times faster than the inactivation rate we observed in mucus. Interestingly, that study also demonstrated accelerated inactivation in simulated saliva when compared to tissue culture media. The reason for this is unclear, since accelerated inactivation was not observed in the simulated saliva in the absence of sunlight. From these findings and our results, it appears that sunlight and matrices can have complex interactions. These results demonstrate that simulated mucus appears to be protective to SARS-CoV-2, perhaps due to the presence of one or more of the three types of protein (high molecular weight proteins, low molecular weight peptides, and mucus material) (8).

Overall, these findings are important in determining plans for the maintenance and decontamination of outdoor spaces as public health measures are relaxed. Next steps should include examining the effect of sunlight on SARS-CoV-2 in other matrices where infectious virus has been recovered.

Declarations

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Figures

Figure 1

Viability of SARS-CoV-2 on stainless steel after exposure to simulated sunlight. SARS-CoV-2 was suspended in (A) culture medium or (B) an organic matrix, deposited on stainless steel, desiccated, and exposed to either simulated sunlight ("Sunlight") or corresponding ambient conditions ("Control"). Graphs in (A) and the top row of (B) show linear regression fits for the titer of viable eluted virus, expressed as the Log10 50% tissue culture infectious dose per milliliter (TCID50/ml), following culture in Vero cells. The limit of quantitation (LOQ), denoted by a dashed line, is 1.5 logs or 3.16×10^1 TCID50/ml. Plots show the mean and SD of 3 biological replicates per time-point, with each biological replicate representing the average of 3 technical replicates. Viability values falling below the LOQ were not quantifiable and therefore excluded from analysis. Graphs in (A) represent heat-controlled experiments where coupon temperature was held constant at 22.5°C (relative humidity was 34%). Graphs in the bottom row of (B) show coupon temperature and relative humidity readings measured at each time-point during heat-permitted assays, and correspond to the experiment represented in the graph located directly above. The viability of SARS-CoV-2 observed after sunlight exposure was observed to be significantly lower than that measured in control experiments under all conditions ($P \leq 0.0045$). The slope of the regression line for

control virus diluted in medium under heat-controlled conditions was not significantly different from zero ($P = 0.849$). The slope, 95% CI, and goodness of fit parameters, r^2 (coefficient of determination) and $Sy.x$ (SE of estimate), for each regression analysis are shown above and below the best-fit curves representing the control and experimental viruses, respectively. All graphs were created and statistical analyses performed using GraphPad Prism software.

Supplementary Files

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- [Appendix20201127.docx](#)