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Effect of UV and violet light on SARS-CoV-2

S. Strizzi[1], M. Biasin[1], A. Bianco[2], A. Macchi[2], O. Utyro[2], M. Lualdi[3], A. Cavalleri[4], G. Pareschi[2] and M. Clerici[5,6]

[1] Department of Biomedical and Clinical Sciences L. Sacco, University of Milan, Milan, Italy
[2] Italian National Institute for Astrophysics (INAF) – Brera Astronomical Observatory, Merate, Italy
[3] Department of Imaging Diagnostic and Radioterapy, IRCCS Foundation, Istituto Nazionale dei Tumori, Milan, Italy
[4] Epidemiology and Prevention Unit, IRCCS Foundation, Istituto Nazionale dei Tumori, Milan, Italy.
[5] Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy.
[6] Don C. Gnocchi Foundation, IRCCS Foundation, Milan, Italy.
[7] e-mail: andrea.bianco@inaf.it

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Abstract. We performed an in-depth analysis of the virucidal effect of UV light on SARS-CoV-2 suspended in liquid medium. UV light was emitted by LEDs; in particular LEDs UV-C (278 nm), UV-B (308 nm), UV-A (366 nm) and violet (405 nm). An infectious titer of SARS-CoV-2 typically found in the sputum of COVID-19 patients was used to perform the tests using the qPCR (quantitative Polymerase Chain Reaction) approach. We verified that the virus can be completely inactivated by all the wavelengths employed with an increase of the dose going from UV-C to violet light. The long UV-wavelengths correspond to solar irradiation reaching the Earth surface. Our data extend previous results showing that SARS-CoV-2 is highly susceptible to UV light and can be used to support the reduction of incidence of SARS-CoV-2 infection seen in the summer season.

Key words. UV light – SARS-CoV-2 – QPCR – virus inhibition

1. Introduction

SARS-CoV-2 was firstly described in Wuhan, China, as the new coronavirus responsible for pneumonia within the scenario of a new disease: COVID-19. It rapidly became a worldwide pandemic responsible for dramatic and unforeseeable health, social and economic consequences Sohrabi et al. (2019). The implementation of disinfection and prevention strategies is important to limit the spread of new infections. In this context, the germicidal effect on bacteria and viruses by UV light illumination has been widely documented for more than 100 years Reed (2010). The most common mechanism consists in direct absorption of the UV-C photon (usually in the 220 - 280 nm range) by the nucleic acid basis and/or capsid proteins leading to the formation of photoproducts that inactivate the virus Wong et al. (2016); Dumyahn et al. (1999). Some

models have been proposed to correlate the nucleic acid structure with the required dose to inactivate the virus, but a reliable model has not been established. The most common and cheap way to produce UV-C light comes from low pressure mercury tubes and they are employed in disinfection of wastewaters and closed environments as well as of blood products (Miller et al. 2013; Wong et al. 2016; Emilio et al. 2021; Dumyahn et al. 1999). More recently, LED technology is becoming appealing because it is environmental friendly, it is possible to dim the light and tune it according to the application Casini et al. (2019). Unfortunately, UV-C LEDs are expensive and the lifetime is much shorter than common visible LED, but the path is clear and we can expect an improvement of these properties in the next years. A key point in the application of UV light to make disinfection devices is the determination of the dose necessary to inhibit the virus or to decrease its concentration of a certain factor (2-log, 3-log, etc.). With inhibition, we refer to the capacity of UV to prevent virus infection/replication Magden et al. (2005). Unfortunately, in the published works, UV-C measurements were conducted using different viruses and diverse experimental conditions. Consequently, an extremely wide range of values for the same virus is obtained. This was observed for SARS-CoV-1 Duan et al. (2003); Walker et al. (2007); Eickmann et al. (2020) for which, the values range from a few mJ/cm^2 to hundreds mJ/cm^2. A similar situation is occurring for SARS-CoV-2. Indeed, it has been shown to be highly sensitive to UV-C light Inagaki et al. (2020); Ruetalo et al. (2021); Heilingloh et al. (2020); Biasin et al. (2021), although discrepancies in the results indicated that all the variables involved in the experimental setting have to been taken into account to obtain reliable and replicable data. The determination of the UV-C doses is necessary for the development of air and surface disinfecting devices, but do not explain the particular seasonal epidemiology of SARS-CoV-2 infection peaking in winter and being greatly reduced in summer. Thus, the idea that viral epidemiology could be modulated by the intensity of the solar irradiation is rebutted by the observation that the UV-C light emitted by the Sun, the most important UV light source, is filtered and blocked by the ozone layer in the stratosphere. UV-A and UV-B radiations, on the other hand, reach the Earth surface with an irradiance dependent on the season, the latitude, and the weather conditions. The effect of solar UV-A and UV-B radiation on microorganisms is reported in the literature (see for example Nelson et al. (2018); Ratnesar-Shumate et al. (2020)) and also the evidences of seasonal behavior of infectious diseases are reported Lytle et al. (2005); Weber et al. (2008). On that basis, recent models suggest that SARS-CoV-2 infection is indeed solar-sensitive Stevenson et al. (2021); Merow et al. (2020). To prove at the laboratory level this effect, it is necessary to perform tests at different UV wavelengths and determine the doses, if they exist, necessary to inhibit the virus replication and support the epidemiology models. For these reasons, we irradiate the SARS-CoV-2 at wavelengths ranging UV-C to violet light with different doses and the replication of the virus is evaluated.

2. Results and Discussion

To examine the efficacy of UV-inactivation in a real-world scenario, we used the UV-doses reported in Table 1 on a SARS-CoV-2 viral concentration equivalent to the one found in the sputum of infected patients ($1.5x10^3$ TCID50/ml) as reported in the literature Bullard et al. (2020). This concentration is representative of a situation where sick people are present and it is conservative in the case of airborne dispersion of droplets containing the virus or in general the virus in aerosol form. The virus replication overtime is evaluated by means of standard techniques and in particular with Real Time quantitative Polymerase Chain Reaction Assays (qPCR). In detail, a viral stock (Virus Human 2019-nCoV strain 2019- nCoV/Italy-INMI1, Rome, Italy) suspended in Dulbecco's Modified Eagle's Medium (DMEM, ECB20722L, Euroclone, Milan, Italy) was placed under the lamp and irradiated with 3 different doses (D1, D2, D3) for each tested wavelength (see Table 1 for the doses values and exposure times). As light

Table 1. Features of the LEDs employed in the experiments: peak wavelength, the average transmittance of the 1 mm thick DMEM solution and the three doses D1, D2, D3 with the corresponding exposure times.

LED	Peak λ	Solution trans.	D1		D2		D3	
	(nm)		(mW/cm^2)	(s)	(mW/cm^2)	(s)	(mW/cm^2)	(s)
UV-C	278	0.81	2	10	4	20	12	60
UV-B	308	0.93	100	315	200	630	600	1890
UV-A	366	0.95	2000	333	4000	666	12000	1998
Violet	405	0.95	4000	479	8000	958	24000	2874

sources, we employed LEDs in order to have the response of the virus at a quasi monochromatic light. Their emission spectrum is reported in Fig. 1. Since the light is partially absorbed by the DMEM as function of the wavelength, the doses used and reported in this paper are those corrected by the attenuating factor. Preliminary tests were also conducted with UV-C light in order to confirm that the virus response was not dependent on the irradiance/time combination, but just on the total dose delivered. After the illumination, VeroE6 cell cultures were incubated with the virus inoculum in quadruplicate for one hour at 37°C and 5 CO2 and viral replication was assessed by qPCR, as previously described Biasin et al. (2021), at 24, 48 and 72 hpi. The unexposed SARS-CoV-2 sample was used as control. The results of the qPCR as function of the illuminating doses and over time are reported in Fig. 2. In particular, we report the number of copies of the viral RNA for two sequences, namely N1 and N2 as already done in a previous work Biasin et al. (2021). It is important to notice that the employed approach provides the dose for a complete inhibition of the virus, but it does not allow to determine the fraction of the virus still active. The TCID50 method is suitable for such kind of evaluation.

Results show that the virus replicates for the lowest doses. Indeed, we see that the number of copies increases with time, reaching values similar to the untreated sample. Increasing

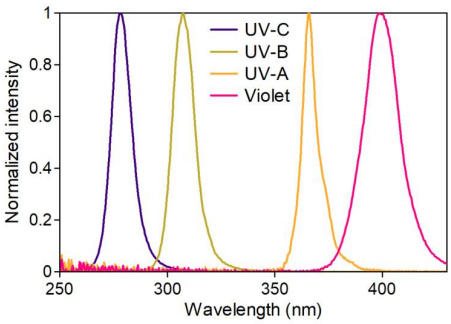

Fig. 1. Normalized emission spectra of the LEDs employed in the illumination of the virus stock. UV-C (278 nm), UV-B (308 nm), UV-A (366 nm), Violet (405 nm).

the dose, in particular to D2 for UV-C, UV-B, UV-A and D3 for the Violet, a complete inhibition of the amplification of the viral genome occurs as apparent from a marked reduction of viral copy number at 24 hour post infection (hpi). Clearly the dose needed for achieving the inhibition increases with the wavelength: 4mJ/cm^2 at 278 nm, 200 mJ/cm^2 at 308 nm, 4000 mW/cm^2 and 24000 mJ/cm^2 at 405 nm. This is not surprising, since the absorption cross-section of both nucleic acid and proteins decreases going from UV-C to visible; therefore the probability of an event that prevents the replication decreases. Such trend has been published in the literature, but with a much smaller effectiveness increasing the

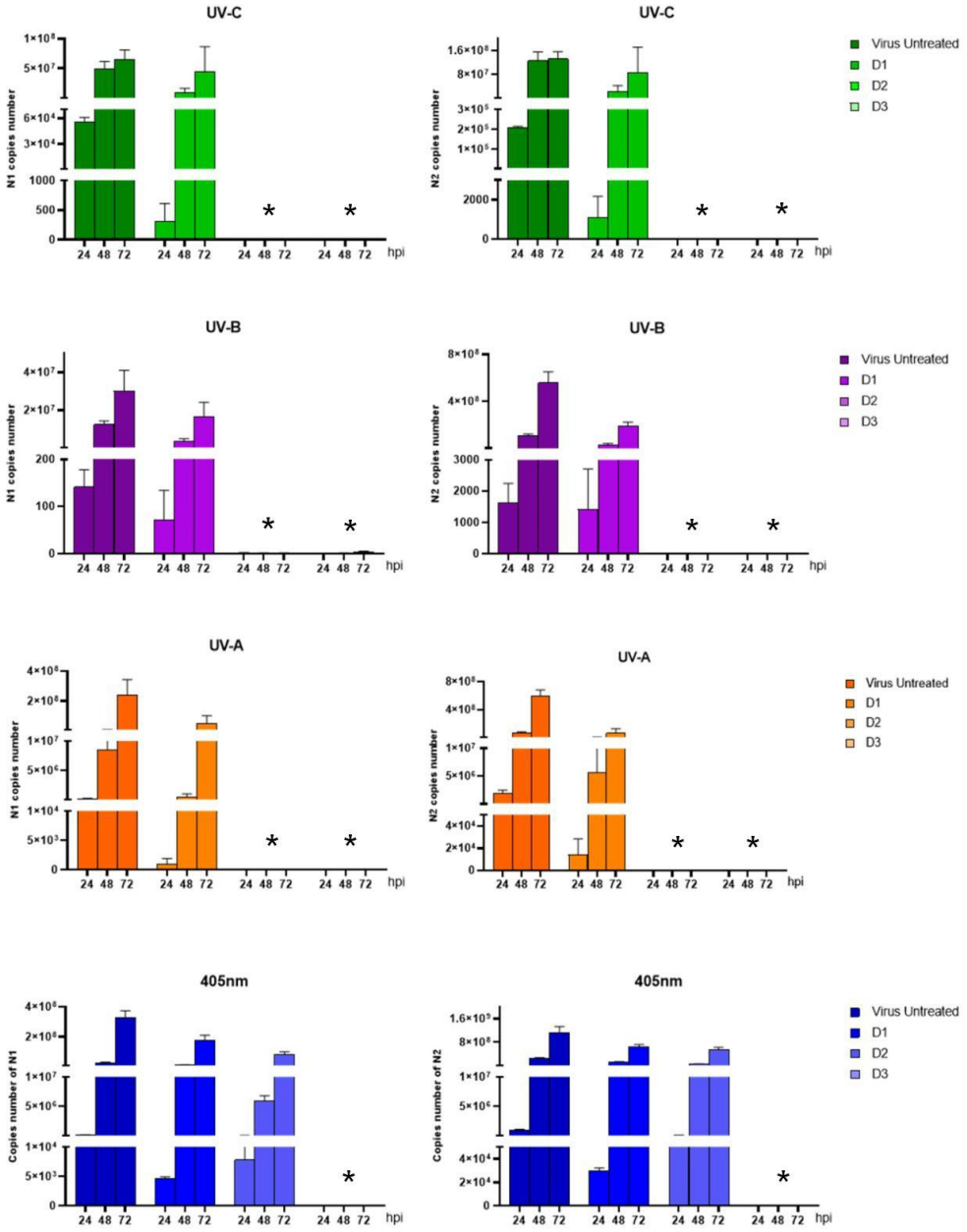

Fig. 2. Viral replication of UV-irradiated SARS-CoV-2 ($1.5x10^3$ TCID50) in the supernatant of in vitro infected VeroE6 cells. Vero E6 cells were infected with SARS-CoV-2 irradiated with different doses (D1, D2, D3) of UV-A, -B, -C and violet light. Culture supernatants were harvested at the indicated times (24, 48 and 72 hpi) and virus titers were measured by absolute copy number quantification (Real-Time PCR). All cell culture conditions were seeded in quadruplicate. Mean values ±SEM are shown. * no bar is shown since the value was below the detection limit.

wavelength. Interestingly, the dose at 278 nm is very similar to the dose we measured at 254 nm Biasin et al. (2021), meaning that the effectiveness of the UV-C light in the 250 - 280 nm range is almost flat. What is important is that also violet light is effective in producing a complete inhibition of the virus replication and this is quite surprising and interesting. Indeed, this is visible light, so much less dangerous for the human being and the content of such wavelengths in the sun emission is much higher than UV-B and UV-A. In addition, our data support (and can be used) the models that predict the disinfection effect of the sun illumination as function of the season.

3. Conclusions

The capability of UV and violet light in the inhibition of the SARS-CoV-2 replication was evaluated in DMEM solution with a viral concentration commonly founded the sputum of SARS-CoV-2 infected patients. The virus is susceptible to all the employed wavelengths. Going from UV-C (278 nm) to violet light (405 nm), the dose required for a complete inhibition. Indeed, 4 mJ/cm^2 are enough at 278 nm that increase to 24000 mJ/cm^2 at 405 nm. The doses for UV-B (200 mJ/cm^2) and UV-A (4000 mJ/cm^2) lie in between. This behavior is similar to that of influenza virus and much different from that of DNA viruses and bacteria, which are not affected by UV-A and violet light. The reported experimental data confirm the high effectiveness of UV light on this virus and they can be useful to support the models explaining the solar effect and the seasonalityNicastro et al. (2021).

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