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Brief Report

Methylene blue in combination with sunlight as a low cost and effective disinfection method for coronavirus-contaminated PPE

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A B S T R A C T

Using the Murine Hepatitis Virus (MHV) A59 coronavirus as a SARS-CoV-2 animal surrogate, we validated that methylene blue (MB) in combination with sunlight exposure is a robust, fast, and low-cost decontamination method for PPE that should be added to the toolbox of practical pandemic preparedness.

INTRODUCTION

COVID-19 has not only highlighted the unpreparedness of the world to handle a global pandemic, but it has also unmasked the inequity of resources available.[1-3] A major issue from the beginning of the pandemic has been the availability of personal protective equipment (PPE), including surgical masks and respirators,[1-3] which still remains problematic in the same areas of the world where vaccines are not readily available to the general public.[2] Consequently, efforts have been made to sanitize and re-use PPE.[4-5] Published sanitization techniques are on the hours' time scale and often require equipment that is not readily available to low-resource settings.[4-5] Therefore, there is a need for cost-effective and time-efficient methods for the safe decontamination of PPE.

A recent global collaboration, which included our group, participated in a study on the Development and Methods for N95 Respirators and Mask Decontamination (DeMaND) and validated methylene blue (MB) in combination with visible indoor light to efficiently disinfect PPE contaminated with infectious SARS-CoV-2 or surrogate animal viruses.[6] Herein, we undertake a follow-up study and demonstrate the efficacy of MB in combination with sunlight to decontaminate surgical masks, solidifying the position of MB as a robust decontamination method for all settings.

MATERIALS AND METHODS

Virus and cell

The murine hepatitis coronavirus (MHV) strain A59 and the delayed brain tumor (DBT) cells were kindly supplied by Dr Alain Lamarre, INRS, Quebec, Canada. All cultures were grown at 37 °C in a humidified incubator with 5% CO_2 according to literature protocols.[7] The infectious activity of the viral cultures was determined in 6-well plates (VWR) by plaque counting assays.[8] MHV stocks with titers ranging from 5.18 ±0.22 $\log_{10}$ pfu/mL to 6.28±0.23 $\log_{10}$ pfu/mL were used in subsequent steps.

Surgical masks

Razor Medical Surgical Disposable Face Masks: ASTM F2100 Level 1 were purchased from IDA pharmacy at the University of Calgary.

Simulated sunlight

Sunlight was simulated with a SolSim 2 photoreactor (Luzchem, Canada) that matches the air mass AM1.5 solar spectrum to within 1% total power difference between 300-800 nm, equivalent to the yearly average irradiance measure at mid-latitude (41.81° above the horizon) on an inclined plane at 37° tilt towards the equator, with a

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solar zenith angle of z=48.2°. The AM1.5 spectrum has an integrated power of 1000W/m^2 (280-4000 nm).

Methylene blue treatment in solution

Solutions of MHV were made at a 10^{-2} dilution in MEM without phenol red (Quality Biological) in the absence (control) and in the presence of 10 μM MB (treated). The solutions, with and without MB, were irradiated under simulated sunlight for various times, except for the solution referred to as time 0 min, which was kept in the dark. Virus inactivation at different irradiation time points was quantified via a plaque counting assay.[8] Integrity of the genetic material was assessed by next generation sequencing (Illumina MiSeq, 300 cycle nano flowcell, paired-end). Viral RNA was extracted from selected samples using a QIAamp Viral RNA kit (Qiagen) according to manufacturer protocol on triplicate samples. Library input was evaluated using D1000 ScreenTape (Agilent). Sequencing libraries were prepared using the Ultra II RNA kit (NEB) with no fragmentation, and adapter ligation efficiency was evaluated using the KAPA Library Quantification Kit (Roche). The raw sequences for the RNA sequencing experiment have been deposited in the NCBI SRA under accession PRJNA806985, while mapping and mutation statistics were generated using scripts and reference datasets available at http://github.com/nodrogluap/damage.

Virus inoculation and elution on a surgical mask

Surgical masks were cut into 1 cm^2 square coupons. A 10 μM aqueous MB solution was sprayed onto several mask cut-outs (treated). Each coupon was placed down on a bench and sprayed 3 times (1 mL/spray) from 10 cm above to ensure full identical coverage of the coupons treated with MB. The coupons were then allowed to dry overnight in the dark, while control coupons were left untreated. To all coupons, 20 μL of the virus was inoculated. After sunlight exposure, the virus was eluted from the mask according to previously developed protocols.[6] Virus inactivation was quantified via plaque counting assay.[6,8]

Data analysis

All data were computed and analyzed using GraphPad Prism 9 (Graph-Pad Software).

RESULTS

As shown in Figure 1A, complete viral inactivation was observed within 5 minutes of sunlight exposure for solutions treated with 10 μM MB, while it required at least 3 hours of sunlight exposure to reach a 50% reduction in MHV log pfu in the absence of MB. The chemical integrity of the viral RNA was assessed via next generation sequencing on triplicate samples. Viral RNA for samples treated with 10 μM MB in combination with 15 minutes of sunlight exposure showed a decreased sequencing library prep yield and poorer adapter ligation efficiency (Mann-Whitney test P=.0091 for both). Furthermore, as shown in Figure 1B, in an equimolar pooling of all libraries, only the sample treated with MB plus sunlight showed a significantly smaller mean fragment size (~239nt vs ~128nt) and much lower percentage mapping to the MHV genome (0.05% for samples treated with MB plus sunlight vs 15.3% for other samples). This is consistent with MB plus sunlight treatment causing severe damage to all RNA backbones, and especially depleting intact viral RNA. Interestingly, no treatment (sunlight exposure and/or presence of MB) consistently caused a significant change in the observation of T mutations in the most common UV-lesion (YCY, n=1375) and 8-oxoG (CGG, n=163) contexts of the MHV reference genome vs corresponding background mutation rates.

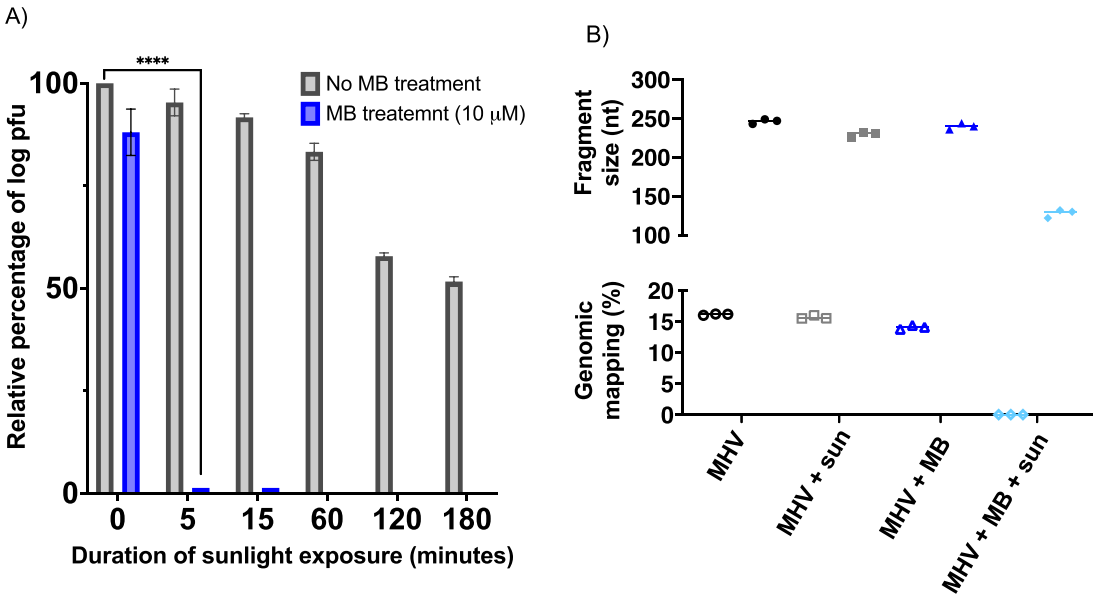

Fig 1. (A) Antiviral activity of MB and/or sunlight exposure against MHV expressed as relative log pfu (%) to that for the untreated virus-infected control cells, which was defined as 100%. The data shown are the mean ± SD from three replicated experiments. P-value<.0001 (****). (B) Next generation sequencing statistical data for the median virus mapped fragment size (nucleotide, nt – top), and reads mapping to MHV-A59 genome (percentage, % - bottom). MHV is an untreated viral sample kept in the dark (control); MHV + sun corresponds to the viral sample irradiated for 180 minutes under simulated sunlight exposure; MHV + MB represents the viral sample treated with 10 μM MB kept in the dark; MHV + MB + sun is the viral sample treated with 10 μM MB in combination with 15 minutes of simulated sunlight exposure.

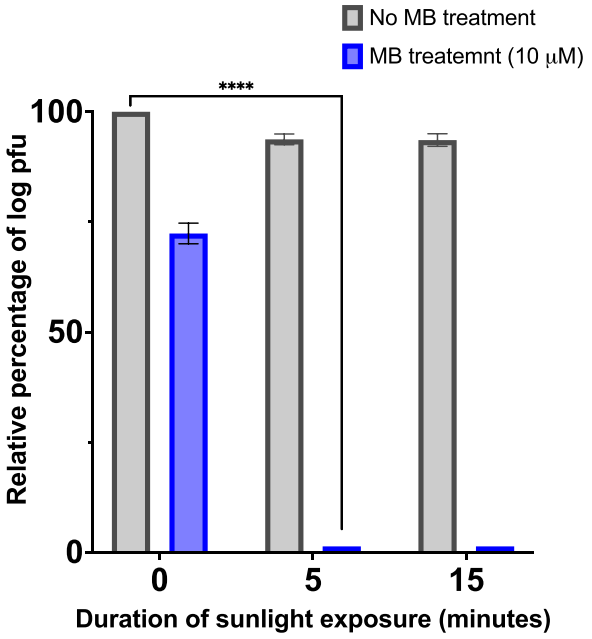

Fig 2. Antiviral activity of MB and/or sunlight exposure against MHV contaminated masks expressed as relative log pfu (%) to that for the untreated virus-infected control cells, which was defined as 100%. The data shown are the mean ± SD from three replicated experiments. *P*-value<.0001 (****).

Importantly, pre-treatment with 10 μM MB resulted in the complete viral inactivation on surgical masks after only 5 minutes of sunlight exposure (Fig 2).

DISCUSSION

As demonstrated in our previous study, methylene blue in combination with bright indoor light (50,000 lux) generated by a white LED lamp consistently inactivates SARS-CoV-2, and other animal surrogates, including MHV, in 30 minutes or less.[6] While this decontamination method is time and cost-effective, it still requires the light source to be powered, and thus it relies on the need for electricity, which remains unavailable to nearly 16% of the world population. Therefore, we set to validate that sunlight can be used to activate MB and disinfect masks contaminated with MHV, a SARS-CoV-2 animal surrogate.[6,9] Sunlight itself has already been reported to inactivate SARS-CoV-2, with time ranging from as little as 8 minutes to several hours.[10-12] Results shown in Figure 1A show that MHV is more resistant to sunlight, making it an ideal surrogate for the combination treatment of MB and sunlight. Herein, we showed the superiority of combining MB with sunlight. Indeed, total inactivation of MHV was achieved within 5 minutes of sunlight irradiation in solution, while it required several hours for sunlight to display antiviral activity (Fig 1A). Interestingly, the observed fundamental genetic damage (low viral RNA proportion, short fragments, and poor ligation efficiency, Fig 1B) when the virus is treated with MB in combination with sunlight exposure strongly suggests that this synergistic approach will also work in depleting viable genomic RNA in other sarbecoviruses such as SARS-CoV-2. In the absence of light, a small reduction of viral titers was also found for samples treated with MB, which was already observed in our past study, and can be attributed to the antiviral activity of MB itself.[6] Importantly, we demonstrated that surgical masks' material contaminated with MHV can be fully decontaminated within 5 minutes of sunlight exposure when treated with 10 μM MB, while significant infectious titers remain on the masks' material exposed solely to sunlight (Fig 2).

CONCLUSION

This brief report validates the use of methylene blue in a combination of sunlight, as a robust decontamination method for PPE, such as surgical masks, that can be deployed in any setting, especially in remote areas where electricity is not readily available.

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