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Investigation of mouse hepatitis virus strain A59 inactivation under both ambient and cold environments reveals the mechanisms of infectivity reduction following UVC exposure

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ARTICLE INFO

Keywords:
Coronavirus
MHV-A59
SARS-CoV-2
Surface contamination
UVC inactivation
Inactivation mechanism

ABSTRACT

The surface contamination of SARS-CoV-2 is becoming a potential source of virus transmission during the pandemic of COVID-19. Under the cold environment, the infection incidents would be more severe with the increase of virus survival time. Thus, the disinfection of contaminated surfaces in both ambient and cold environments is a critical measure to restrain the spread of the virus. In our study, it was demonstrated that the 254 nm ultraviolet-C (UVC) is an efficient method to inactivate a coronavirus, mouse hepatitis virus strain A59 (MHV-A59). The inactivation rate to MHV-A59 coronavirus was up to 99.99% when UVC doses were 2.90 and 14.0 mJ/cm² at room temperature (23 °C) and in cold environment (–20 °C), respectively. Further mechanistic study demonstrated that UVC could induce spike protein damage to partly impede virus attachment and genome penetration processes, which contributes to 12% loss of viral infectivity. Additionally, it can induce genome damage to significantly interrupt genome replication, protein synthesis, virus assembly and release processes, which takes up 88% contribution to viral inactivation. With these mechanistic understandings, it will greatly contribute to the prevention and control of the current SARS-CoV-2 transmissions in cold chains (low temperature-controlled product supply chains), public area such as airport, school, and warehouse.

1. Introduction

The outbreak of COVID-19 has been causing a global pandemic since December 2019. It has spread across 224 countries and territories, and resulted in about 282 million confirmed cases thus far according to WHO Coronavirus (COVID-19) Dashboard [1]. In addition to the airborne transmission of the SARS-CoV-2 viruses [2], the surface contamination has been becoming a potential issue, and several infection incidents have been reported [3–8]. Studies have shown that viruses can remain infectious on surfaces for days [3,9], and surfaces of common items in daily life, e.g., plastics, glass, cold chain packaging, etc., are vulnerable to contamination by viruses [10,11]. In cold chain industry, the whole process, i.e., production, packaging and transportation, are generally performed at lower temperatures [12], this further creates a favorable environment for the survival of SARS-CoV-2 on the contaminated surface [13–16]. When items with contaminated surfaces were transported, it even facilitated the spread of virus domestically or internationally [17, 18]. Since the sampling and screening of the viruses by nucleic acid assay are quite labor intensive, the disinfection of contaminated surfaces is an effective and critical measure to restrain the spread of the virus.

SARS-CoV-2 is single-stranded RNA coronavirus [19]. For disinfection, the use of liquid disinfectants, e.g., isopropyl alcohol (2-propanol), chlorine dioxide, quaternary ammonium compounds (QACs), and hydrogen peroxide, and heat treatment, have been proved to be quite effective.
effective to reduce the infectivity of SARS-CoV-2 [20–24]. However, above methods have limitations, and they are not effective for the surfaces of large items, e.g., packages in cold chain, warehouse, etc. Ultraviolet-C (UVC), with radiation wavelength in the range of 200–280 nm, has been demonstrated as an efficient method for disinfection of pathogens on surfaces, air and water [25]. It could be used for large area disinfection with the advantages of high efficiency, saving energy and better maintaining the quality of frozen products compared with high-temperature treatment [26–30]. In addition, it is a safe inactivation without chemical residues compared with using disinfectants [29,30]. Studies have demonstrated that UVC irradiation can restrain the viable SARS-CoV-2, and even high concentrations of viral stock at 5 × 10⁶ Tissue Culture Infectious Dose 50% (TCID50)/mL were completely inactivated by UVC [31,32]. However, there is no study on the effectiveness of UVC irradiation on inactivation of viruses residing in ice, and the mechanism of UVC inactivating coronavirus still remains unknown. Therefore, we herein try to investigate the disinfection ability of 254 nm UVC against a mouse coronavirus (MHV-A59), a coronavirus in the same coronavirus genus as SARS-CoV-2 [33,34], and further explored its inactivation mechanism. It is expected to provide help to the prevention and control of the current COVID-19 pandemic or other possible infectious pathogens.

2. Materials and methods

2.1. Materials

MHV-A59 viruses (ATCC VR7-64) and L929 cells were purchased from American type culture collection (Maryland, America). Dulbecco’s Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Wisent Biotechnology (Nanjing, China). Phosphate-buffered saline (PBS) was purchased from Solarbio (Beijing, China). Virus DNA/RNA Kits were purchased from TGuide (Beijing, China). One Step PrimeScript™ RT-PCR Kits were purchased from TaKaRa Bio (Kyoto, Japan). The protocols of MHV-A59 infection and reverse transcription (RT)-quantitative real-time polymerase chain reaction (qPCR) assay were approved by Guangdong Laboratory Animals Monitoring Institute.

2.2. The irradiation system setup and 254 nm UVC dose determination

The irradiation system was set as Fig. 1A and B. In the biosafety cabinet with dark environment, the 254 nm UVC light (LEC-280L, LUYOR Instrument, China) vertically irradiated the samples or the spectrometer sensor (ILT950 with W/A2 UV-NIR SINGLE CAL, A&P Instrument, USA) placed right below the light source. The final
irradiation intensity was recorded. By change the distance from the light source to the sensor, the UVC dose was calculated by the below equation [27,31,32]:

\[
\text{UVC dose (mJ/cm}^2\text{)} = \text{Irradiation intensity (mW/cm}^2\text{)} \times \text{Exposure time (s)}
\]

The different UVC irradiation distances, intensities, exposure times and UVC doses used in this study were showed in Table S1. Because exposure time is a better-controlled condition than UVC intensity, we can change exposure times (from 1 s to 120 s) among intensities to acquire a wide range of UVC doses from 0, 0.105, 0.290, 2.90, 14.0, 83.7 to 107 mJ/cm².

### 2.3. The penetration ability of 254 nm UVC light in frozen PBS

PBS solution was added into a culture dish and frozen overnight at –20 °C. The thickness of frozen PBS was 6 mm. The frozen PBS was removed from the culture dish and placed on the spectrometer. The 254 nm UVC light vertically penetrated the frozen PBS, and the spectrometer sensor was placed right below the light source (Fig. 1A). The irradiation intensities before and after the penetration of frozen PBS were compared, and the energy loss was shown in Table S2.

### 2.4. The determination of MHV-A59 infectivity before and after UVC treatment with different doses at room temperature (23 °C) and in cold environment (–20 °C)

0.1 mL of MHV-A59 stock solution (2.95 × 10¹⁰ pfu/mL) was diluted with 0.9 mL of PBS in culture dishes. The diluted solution was rolled out on the culture dish (R = 9 cm) at room temperature (23 °C), and the thickness was less than 2 mm. Then, the solution was treated by UVC with different doses i.e., 0, 0.105, 0.290, 2.90, 14.0, 83.7 and 107 mJ/cm², and the viruses were collected. The virus titers were determined by Tissue Culture Infectious Dose 50% (TCID50) assay [12,35]. Briefly, different UVC-treated virus solutions were diluted 1–10⁹ folds with DMEM. In each group, 100 μL of virus solutions with different dilutions were mixed with 100 μL of 6.5 × 10⁴ L929 cells. The CPE on L929 cells was observed and recorded. The CPE results were used to calculate virus titers by Spearman-Karber method [36]. The fractions of infectious MHV-A59 virus after UVC irradiation with different doses were calculated by using virus titers. The raw data of virus titers in different treatment groups were showed in Table S3.

To determine the infectivity of MHV-A59 before and after UVC irradiation in cold environment, 0.1 mL of MHV-A59 stock solution (2.95 × 10¹⁰ pfu/mL) was diluted 10 folds with PBS in culture dishes, the diluted virus solutions were frozen at –20 °C for 0.5 h. Then, they were immediately irradiated by UVC with different doses (0, 0.105, 0.290, 2.90, 14.0, 83.7 and 107 mJ/cm²) and then collected in tubes. The raw data were also shown in Table S3.

### 2.5. The genome loss in each step during viral infection process following UVC irradiation

The experimental methods to detect genome loss during virus infection process was optimized from the studies of Wigginton et al. [28] and Rattanakul & Oguma [37]. The experimental conditions such as incubation temperature, time and wash buffer used for removing MHV-A59 virus attached to the host cells were optimized, the experimental details were shown in Method S1, Table S4 and Fig. S3.

The specific details on the detection of genome loss in each step determined by pre-optimization experiments were shown as the methods section of Supplementary Materials (Method S1). The methods were briefly stated here.

#### 2.5.1. RT-qPCR assay after UVC treatment

Viruses were collected after treated by UVC (0 and 107 mJ/cm²).

The genome loss was measured by RT-qPCR. To isolate MHV-A59 RNA from viral suspension, a TGuide Virus DNA/RNA Kit and an automatic nucleic acid extraction instrument (TGuide, Beijing, China) were used after three freeze-thaw cycles. Two gene segments derived membrane (M) and nucleocapsid (N) proteins were detected as target genes. Their primers and probes were designed and optimized by Guangdong Laboratory Animals Monitoring Institute, and the information was shown in Table S5. According to the protocol of One Step PrimeScript™ RT-PCR Kit, the reaction was performed by incubating 20 μL of reaction mixture including 2 μL of viral RNA, 0.4 μL of PCR forward primer (10 μM) and PCR reverse primer (10 μM), 0.8 μL of probe and other components at 42 °C for 5 min and 95 °C for 10 s in an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystem Japan Ltd., Japan). PCR reaction was repeated for 40 times with the condition of 95 °C for 10 s and 60 °C for 34 s.

The log of genome reduction, I⁡gdetected damage was calculated by the Eq. (2),

\[
I_{\text{detected damage}} = \log_{10}(N_0/N_1)
\]

N₀ and N₁ were the detected gene copies of MHV-A59 virus treated by UVC (0 and 107 mJ/cm²), respectively. The raw data were shown in Table S6.

#### 2.5.2. Virus attachment assay after UVC treatment

MHV-A59 viruses were mixed with L929 cells at a multiplicity of infection (MOI) of 1 after being treated by UVC (0 and 107 mJ/cm²). The mixed samples were incubated in 1 mL of cold PBS (4 °C) for 1.5 h. After centrifuged at 10,000 rpm for 10 min and washed with PBS for 3 times, the viruses attached to L929 cells were collected. The RNA was extracted and quantified by RT-qPCR assay.

The log of genome reduction, I⁡gattachment was calculated by Eq. (3), and the result was shown in Table S4.

\[
I_{\text{attachment}} = \log_{10}(N_0/N_1)
\]

N₀ and N₁ were the gene copies of MHV-A59 attached to L929 cells after treated by UVC (0 and 107 mJ/cm²), respectively.

#### 2.5.3. Viral genome penetration assay after UVC treatment

MHV-A59 viruses were mixed with L929 cells (MOI = 1) after treated by UVC (0 and 107 mJ/cm²). The mixed samples were incubated in 1 mL of cold PBS (4 °C) for 1.5 h. After centrifuged at 10,000 rpm for 10 min and washed with PBS for 3 times to allow the genome penetration. The attached viruses were removed by washing L929 cells with PBS containing 0.02% sodium dodecyl sulfate (SDS) for 3 times and PBS for 2 times. The virus genome in the host cells was collected for RT-qPCR assay.

The log of genome reduction, I⁡gpenetration was calculated by Eq. (4).

\[
I_{\text{penetration}} = \log_{10}(N_0/N_1)
\]

N₀ and N₁ were the gene copies of MHV-A59 in the host cells after treated by UVC (0 and 107 mJ/cm²), respectively. The raw data were shown in Table S6.

#### 2.5.4. The calculation method of each process during virus infection

According to the methods by Wigginton et al. and Rattanakul et al., the total number of lost gene copies contributed to the loss of infectivity (I⁡gloss) caused by the interruption of attachment (I⁡gattachment), genome penetration (I⁡gpenetration), genome replication, protein synthesis and virus assembly and release (I⁡greplication) was shown as:

\[
I_{\text{loss}} = I_{\text{attachment}} + I_{\text{penetration}} + I_{\text{replication}}
\]

I⁡gloss was total genome reduction measured by infectivity assay (TCID50 assay), it was expressed as:

\[
I_{\text{loss}} = \log_{10}(N_0/N_1)
\]
that the fraction of infectious virus rapidly decreased with the increase of virus load on contaminated surfaces, which was estimated to be less than $10^2$ gene copies/cm$^2$ on the surface of culture dish, which was calculated from RT-qPCR assay (Table S3). The minimum values of infectious virus fraction in different groups (imaginary lines) were determined by their original titers and lower limit of quantification of TCID50 assay. Error bars indicated standard deviation of 3 independent experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to UVC treatment at room temperature.

By changing UVC dose irradiated to the virus at room temperature ($23 \pm 0.8^\circ$C) and $-20^\circ$C, the virus titers were examined (Table S1), and the virus titers of untreated and UVC-treated groups.

Finally, $lg_{\text{estimated damage}}$ can be expressed in the following equation:

$$lg_{\text{estimated damage}} = lg_{\text{attachment}} - lg_{\text{penetration}} - lg_{\text{detected damage}}$$

2.6. Statistical Analysis

The values were expressed as mean $\pm$ SD. Two-tailed Student’s $t$-test was used to determined statistical significance for analysis between two groups.

3. Results and discussion

3.1. The inactivation rates of UVC treatment to MHV-A59 coronavirus under both ambient and cold environments

The mouse hepatitis virus, MHV-A59, was selected as a surrogate mouse virus model for SARS-CoV-2 disinfection [38–41]. Both MHV-A59 virus and SARS-CoV-2 belong to beta-coronavirus, and their structure and behavior in environment was similar [33,34]. A 254 nm UVC lamp was chosen as light source because of its high virus inactivation capability [42,43], the UV spectrum and irradiation system setup were shown as Figs. S1A and 1, respectively. TCID50 assay was used to determine the infectivity of MHV-A59 before and after UVC treatment. The selected model virus concentration was beyond $5 \times 10^9$ gene copies/cm$^2$ on the surface of culture dish, which was calculated from RT-qPCR assay (Table S6). This titer was much higher than the possible virus load on contaminated surfaces, which was estimated to be less than $2 \times 10^6$ gene copies/cm$^2$ [44]. Furthermore, virus could remain viable for 2 years at $-20^\circ$C [45], thus, it’s necessary to determine if UVC can effectively inactivate the virus residing in this cold environment. It was found that 254 nm UVC could penetrate a layer of 6 mm frozen PBS with energy loss of less than 20% (Table S2) and without peak shift (Fig. S1B). Thus, it suggested that the existence of frozen PBS layer would not significantly influence MHV-A59 inactivation through stopping UVC irradiation. In order to detect virus inactivation under cold environment, the MHV-A59 virus solution was frozen at $-20^\circ$C with a thickness of 2 mm, mimicking a cold chain environment.

By changing UVC dose irradiated to the virus at room temperature ($23 \pm 0.8^\circ$C) and $-20^\circ$C, the virus titers were examined (Table S1), and the fractions of infectious MHV-A59 virus were shown as Fig. 2. It was found that the fraction of infectious virus rapidly decreased with the increase of UVC dose whether at room temperature or $-20^\circ$C. However, the fraction of infectious MHV-A59 virus at $-20^\circ$C, i.e., $(1.34 \pm 0.69) \times 10^2$ 1, was significantly higher than that at room temperature, i.e., $(3.08 \pm 0.00) \times 10^2$, when UVC dose was 2.90 mJ/cm$^2$. In addition, the fraction of infectious MHV-A59 virus was lower than $10^{-4}$ when the UVC dose was 2.90 mJ/cm$^2$ at room temperature but 14.0 mJ/cm$^2$ at $-20^\circ$C. It was found that several-fold higher UVC dose at $-20^\circ$C was needed when the inactivation rate of UVC reached up to 99.99%. It was because that MHV-A59 virus inactivation by UVC was mainly caused by RNA chemical damage, particularly photo-dimerization of proximal pyrimidines [46,47]. However, it was inhibited in cold temperature [48,49]. Calculated by Eq. S1, the UVC susceptibility constant for the MHV-A59 was 0.454 mJ/cm$^2$ at room temperature, and it was 0.159 mJ/cm$^2$ at $-20^\circ$C (Fig. S2). The former was three times as much as the UVC susceptibility constant for SARS-CoV-2, which was calculated to be about 0.135–0.162 mJ/cm$^2$ (MOI = 5) [27]. Besides, UVC dose for 90% inactivation (D90 value) of MHV-A59 and SARS-CoV-2 were 21.0 and 21.5 J/m$^2$, respectively [50]. These results suggested that the UVC sensitivity for MHV-A59 is highly similar to SARS-CoV-2. All together, these results demonstrated that UVC could efficiently inactivate MHV-A59 virus at room temperature and even in cold environment, which may be supposed to be applicable for SARS-CoV-2 because the two viruses belong to beta-coronavirus and are similar in structure and UVC susceptibility.

3.2. The inactivation mechanism of UVC to MHV-A59 virus

Based on the fact that UVC could inactivate MHV-A59 coronavirus, the inactivation mechanism was further explored. It has been demonstrated that coronaviruses could infect host cells through the following steps [38,51], (1) attachment of virus to host cells through binding between viral spike proteins and host-cell receptors, (2) entry or penetration of viral genome to host cells through viral fusion, (3) replication of viral genome in host cells, (4) protein synthesis, (5) virus assembly and (6) virus release from the host. During infection, viral proteins in capsid played an important role during viral attachment and genome penetration, whereas viral genome was vital for the following genome replication, protein synthesis and virus assembly. MHV-A59 virus was treated by UVC with a dose of 107 mJ/cm$^2$ to ensure the killing of live
viral genome reduction during genome replication to virus release processes (lg_{estimated damage}) could be estimated to be about 2.80 (Eqs. 7–9). Furthermore, the detected results from GS2 in each assay were similar to that from GS1 (Fig. 3A and Table S6). In order to quantify how UVC treatment could affect the MHV-A59 virus infection, the loss of infectivity in each infection process was determined based on the viral genome reduction (Fig. 3B), the calculation methods were shown as method 2.4.4 [37]. The total infectivity assay (lg_{infectivity}) was regarded as 100% loss of infectivity. As for the attachment process detected with GS1, the genome reduction (lg_{attachment}) was the difference between genome penetration assay (lg_{penetration}) and attachment assay (lg_{attachment}) (Eq. 11). The lg_{penetration} contributed to 4.4% loss of infectivity (Fig. 3B). It suggested that spike (S) proteins, which were responsible for attachment and genome penetration of coronavirus [53, 54], were damaged by UVC. It agreed with previous study that proteins could be denatured following 254 nm UVC treatment [55,56]. Therefore, it could be expected that S protein denaturation would be induced by UVC irradiation to inhibit viral attachment and genome penetration processes. The loss of infectivity during genome replication to virus release (lg_{replication}) could be associated with detected genome damage (lg_{detected damage}), undetected genome damage during post-replication processes (lg_{estimated damage}) (Eq. 7). The former (lg_{detected damage}) and the latter (lg_{estimated damage}) contributed to 26.0% and 62.2% loss of infectivity, respectively. Furthermore, the detected results from GS2 showed highly similar percentages in each assay with GS1. The sensitivity of the RT-qPCR method depends on the location and size of designed oligonucleotide sequences for genome damage [37]. A long-range PCR would be more appropriate to assess UV-induced genome damage [57]. When a short target genome segment was used, the detected genome damage may be underestimated, as shown in this study. It could be an important reason for the value of estimated genome damage contributed the higher loss of infectivity.

Based on the above data, a potential mechanism of UVC inactivation was proposed in Fig. 3C. It was shown that the virus attachment and the

![Fig. 3. The mechanism of UVC inactivating MHV-A59 coronavirus. A) The log of genome reduction detected by RT-qPCR assay of GS1 and GS2 (gene segments from M protein and N protein, respectively) during virus infection process. B) The contribution of function loss during virus infecting host cells following UVC treatment. C) The inactivation mechanism of UVC to MHV-A59 virus. Error bars indicated standard deviation of at least 4 independent experiments. * * * p < 0.001.](image-url)
genome penetration ability was decreased, which was probably caused by viral S protein denaturation. Besides, genome replication, protein synthesis, virus assembly and release were significantly interrupted by genome damage. The genome damage was a more important factor to induce the inactivation of MHV-A59, which agreed with previous studies that UVC inactivated virus through damaging virus genes [46].

4. Conclusions

In our study, we demonstrated that the 254 nm UVC is an efficient method to inactivate MHV-A59 (99.99%) at room temperature (23 °C) and in cold environment (20 °C) under the doses of 2.90 and 14.0 mJ/cm², respectively. It was further elucidated that UVC could inactivate coronavirus through genome damage and possible S protein denaturation. With these understandings, further work will be employed to translate the demonstrated results for the inactivation of SARS-CoV-2 in the cold chain.

CRediT authorship contribution statement

Bingbing Sun, Changying Xue, Qi Song and Min Li: Conceptualization and Methodology. Min Li, Yunlong Yang, Wenhui Liu, Jiahuan Li and Zhihui Liu: Investigation and Resources. Guanyu Ding, Xiaohie Chen, Changying Xue and Qi Song: Software and Validation. Min Li, Yunlong Yang and Wenhui Li: Formal analysis and Data curation. Min Li, Yunlong Yang and Jiahuan Li: Writing – original draft. Bingbing Sun, Changying Xue and Qi Song: Writing – review & editing. Bingbing Sun: Supervision and Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31870919), LiaonIng Revitalization Talents Program (XLYC1807113), and Dalian Science and Technology Innovation Emergency Fund. We would like to thank Dr. Feng Cong and Ms. Yujun Zhu at Guangdong Laboratory Animals Monitoring Institute for helping with the virus disinfection study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2022.107206.

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