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Short communication

Ultraviolet-C efficacy against a norovirus surrogate and hepatitis A virus on a stainless steel surface

Shin Young Park, An-Na Kim, Ki-Hoon Lee, Sang-Do Ha *

School of Food Science and Technology, Chung-Ang University, 72–1 Nae-Ri, Daeduck-Myun, Ansung, Kyunggido 456–756, Republic of Korea

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ABSTRACT

In this study, the effects of 10–300 mWs/cm^2 of ultraviolet radiation (UV-C) at 260 nm were investigated for the inactivation of two foodborne viruses: murine norovirus-1 (MNV-1; a human norovirus [NoV] surrogate) and hepatitis A virus (HAV). We used an experimentally contaminated stainless steel surface, a common food-contact surface, to examine the effects of low doses of UV-C radiation on MNV-1 and HAV titers. The modified Gompertz equation was used to generate non-linear survival curves and calculate d_R-values as the UV-C dose of 90% reduction for MNV-1 ($R^2 = 0.95$, RMSE = 0.038) and HAV ($R^2 = 0.97$, RMSE = 0.016). Total MNV-1 and HAV titers significantly decreased ($p < 0.05$) with higher doses of UV-C. MNV-1 and HAV were reduced to 0.0–4.4 and 0.0–2.6 $\log_{10}$PFU/ml, respectively, on the stainless steel surfaces by low-dose UV-C treatment. The d_R-value, 33.3 mWs/cm^2 for MNV-1 was significantly ($p < 0.05$) lower than 55.4 mWs/cm^2 of HAV. Therefore, the present study shows that HAV is more resistant to UV-C radiation than MNV-1. These data suggest that low doses of UV-C light on food contact surfaces could be effective to inactivate human NoV and HAV in restaurant, institutional, and industrial kitchens and facilities.

1. Introduction

Norovirus (NoV), hepatitis A virus (HAV), rotavirus, sapovirus, and astrovirus are all major pathogens that cause foodborne illnesses in the United States (Scallan et al., 2011). Among the enteric viruses, NoV is the leading cause of non-bacterial gastroenteritis and is associated with 80 to 90% of reported outbreaks in both developing and developed countries (Glass et al., 2009). HAV infections are also a leading cause of foodborne disease outbreaks occurring regularly in both developing and developed countries (Koopmans and Duizer, 2004; Richards, 2001).

While NoV belongs to the *Caliciviridae* family and HAV belongs to the *Picornaviridae* family, both viruses are primarily transmitted via the fecal–oral route, either by direct person-to-person contact or ingestion of contaminated food and water. Together, NoV and HAV infections are considered to be the most common causes of non-bacterial gastroenteritis, a disease largely resulting from contaminated shellfish (Woods and Burkhardt, 2010; Wright et al., 2009), fresh produce (Butot et al., 2009; Donnan et al., 2012; Heaton and Jones, 2008; Lynch et al., 2009), and ready-to-eat food (Malek et al., 2009; Schmid et al., 2007) around the world. The transmission of pathogenic microorganisms to food via contaminated surfaces is a significant problem in food processing, catering, and domestic environments. Specifically, food-contact surfaces, which are typically made of stainless steel, polypropylene, glass, or wood are used in food processing plants and kitchens. These surfaces can be contaminated as a result of poor personal hygiene, leading to the transfer of the virus to various food products (D'Souza et al., 2006). Among these food-contact surfaces, stainless steel is widely used in food manufacturing and processing industries for manufacture, bulk storage and transportation, preparation, and presentation applications. The use of disinfectants is a key intervention measure to interrupt the transmission of pathogenic microorganisms, including NoV and HAV, onto food-contact surfaces.

The U.S. Food and Drug Administration (FDA) has approved the use of UV-C on food products for controlling surface microorganisms (US FDA, 2007). UV-C radiation in the wavelength range 250–280 nm is normally used to disinfect water, cooking utensils, and liquid foods (Guerrero-Beltrán and Barbosa-Cánovas, 2004). This is especially ideal because UV-C produces no unpleasant odor and has a low energy requirement when applied to foods, utensils, and water. Moreover, UV-C radiation is known to possess antiviral activity in food and water (Fino and Kniel, 2008; Ko et al., 2005; Lenes et al., 2010; Nwachuku et al., 2005; Park et al., 2011) and food contact and swine farm surfaces (Dee et al., 2011; Li et al., 2011). After studying the effects of UV-C on feline calicivirus (FCV; a NoV surrogate) and HAV, Fino and Kniel (2008) observed that UV radiation at 40 mWs/cm^2 reduced viral titer by more than 3 log in lettuce and by less than 1.5 log in strawberries. Li et al. (2011) reported that UV light induced a considerable higher reduction (3 log cycle) of murine norovirus-1 (MNV-1) and bacteriophages as a NoV surrogate on stainless steel. In the study of Jean et al. (2011), pulsed

* Corresponding author at: Dept. of Food Science and Technology, Chung-Ang University, 72–1 Nae-ri, Daeduk-myun, Ansung, Gyunggido 456–756, Republic of Korea.
E-mail address: sangdoha@cau.ac.kr (S.-D. Ha).

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UV light at 60 mWs/cm^2 and 91 mWs/cm^2 induced complete reduction (5 log cycle) of MNV-1 and norovirus-1 and HAV, respectively on stainless steel. Pulsed UV light is more effective and rapid infection of inactivating microorganisms than conventional (continuous) UV light (Jean et al., 2011) whereas this radiation technology is expensive (Choudhary and Bandla, 2012). It is well predictable because it depends on the amount of energy delivered and absorbed by the sample. In this respect, pulsed UV light is not different from continuous UV light.

There is a need to further examine the disinfection effects of conventional UV-C light against foodborne enteric viruses, including NoV and HAV, specifically on food-contact surfaces because this conventional UV light is environmentally friendly, easy to handle and cost-efficient. Therefore, the present study was to investigate the effectiveness of UV-C radiation (10–300 mWs/cm^2) at 260 nm in controlling the infectivity of NoV, using murine norovirus-1 (MNV-1) as an NoV surrogate and an HAV strain on a stainless steel surface, a widely used food-contact surface that could be a major source of cross-contamination and foodborne enteric viruses and was to compare reduction responses of the viruses on the UV-C treated surface.

2. Materials and methods

2.1. Virus cell culture

Murine norovirus-1 (MNV-1; a surrogate for NoV) and Hepatitis A virus (HAV) strain HM-175 were maintained in murine RAW 264.7 cells and monkey FRhK-4 cells, respectively. Cells were cultured in Dulbecco's minimum essential medium (DMEM; Sigma, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 44 mM sodium bicarbonate (Sigma, USA), and 1% antibiotic–antimycotic (Penicillin Streptomycin; Gibco, USA), and seeded into 75 cm^2 culture flasks for incubation at 37 °C in a humidified incubator containing 5% CO_2. The cells were subcultured every two or three days.

2.2. Virus preparation

When the RAW 264.7 and FRhK-4 cell monolayers achieved 90% confluency, the RAW 264.7 cell monolayers were detached using a scraper and FRhK-4 cell monolayers were detached with trypsin. These cells were transferred into a 150-cm^2 culture flasks for viral infection. The growth medium was removed by aspiration and the monolayers were washed with phosphate-buffered saline (PBS, pH 7.4). A 200-μl aliquot of the MNV-1 and HAV HM-175 inoculums was added to the flasks, which were incubated for 30 min to allow virus adsorption. The flasks then received 15 ml of maintenance medium (DMEM + 2% FBS + 44 mM sodium bicarbonate + 1% antibiotic–antimycotic), and were incubated at 37 °C in a 5% CO_2 atmosphere for 3 days (RAW 264.7) or 7 days (FRhK-4). If the observed cytopathic effects were greater than 90%, the virus-infected flasks were frozen and thawed three times. The viruses were released by cell lysis during this step. The above contents were centrifuged at 1500 ×*g* for 10 min to remove the cell debris, and the supernatants were subsequently harvested. The viruses were stored at −70 °C until further use.

2.3. Preparation of stainless steel surface material and inoculation

Stainless steel (Posco Co., Ltd., SUS 304 2B; Pohang, Korea) was selected as a representative material used in the food industry. Stainless steel coupons measuring Ø 10 mm in diameter and 5 mm in thickness were purchased from a Chung-Ang Scientific Inc. (Seoul, Korea). The coupons were soaked in 70% ethanol for 1 h to remove any residue such as oil and then washed with distilled water. After rinsing, the coupons were dried in a desiccator and placed in a sealed bottle to be autoclaved at 121 °C for 15 min. Fifty microliters of each viral suspension, containing approximately 6.20 log plaque-forming units (PFU)/ml of MNV-1 and 5.85 log PFU/ml of HAV, was inoculated onto the surface of the stainless steel coupons using a micropipette. Inoculated coupons were dried in a laminar flow hood for 1 h. Coupon inoculations were performed in triplicate.

2.4. UV-C radiation

UV radiation experiments were performed as described by Ha et al. (2009) to evaluate the effect of UV-C on MNV-1 and HAV on the surface of a stainless steel coupon. A bench-scale, collimated-beam UV reactor equipped with 10-, 15-, and 30-W low-pressure UV lamps (Sankyo Ultraviolet Co., Ltd.; Seoul, Korea) emitting monochromatic UV-C radiation at 260 nm was used in these experiments. UV radiation exposure was measured with an HD 2102.2 photo radiometer (Daehyuntech Co.; Seoul, Korea). The photo radiometer was placed at the same distance (15 cm) as the sample from the UV lamp and calibrated at 260 nm. UV exposure was set at 10, 20, 30, 40, 50, 60, 90, 120, 180, 240, and 300 mWs/cm^2. The applied UV dosage (mWs/cm^2) was calculated as the exposure time (s) multiplied by the adjusted UV irradiance (μWs/cm^2). The UV lamp was turned on at least 30 min prior to the experiments to ensure a constant UV intensity output.

2.5. Sample processing for virus recovery

Following UV-C radiation, 50 μl of maintenance medium (DMEM + 2% FBS + 44 mM sodium bicarbonate + 1% antibiotic–antimycotic) were deposited on the center of UV-C irradiated stainless steel coupon. The virus-contaminated stainless steel coupon containing the maintenance medium was soaked in 450 μl of the medium in a 15-ml conical tube. The samples were vortexed for 2 min to elute the virus. Each eluted viral suspension was serially diluted 10-fold in DMEM. Stainless steel coupon not expose to UV-C was processed exactly the same way. $\log_{10}$ reduction values were calculated as follows: $\log_{10}$ reduction = $\log_{10}$ virus titer for the untreated control − $\log_{10}$ virus titer after treatment. Prior to the inactivation study, the amount of viral particles eluted from the surface of stainless steel coupon processing method was determined and considered for calculation of the $\log_{10}$ reduction in inactivation studies. The recovery percentage for each virus was calculated as % recovery = $[(T_i / T_e) \times 100]$, where T_i = initial viral titer in suspension and T_e = vital titer after elution. Viral titers were determined with a plaque assay.

2.6. Virus titration

The MNV-1 and HAV titrations were performed as previously described, with minor modifications as suggested by Wobus et al. (2004) and Bidawid et al. (2000), respectively. Briefly, confluent RAW 264.7 cells or FRhK-4 cells were seeded into each well of a 12-well plate and incubated at 37 °C with 5% CO_2 for 36 h (RAW 264.7) or 72 h (FRhK-4) to reach 90% confluence. Viral suspensions eluted from the samples were serially diluted in the maintenance medium (DMEM + 2% FBS + 44 mM sodium bicarbonate + 1% antibiotic–antimycotic). Serially diluted viral suspensions (100 μl) were used to inoculate the cells. After shaking the plates for 10 min (FMS2, FINEPCR, Korea), they were incubated at 37 °C with 5% CO_2. One hour later, 2× Type II agarose (Sigma) supplemented with 2× DMEM was added to the inoculated cells; each well received 1 ml of the mixture. The above plates were left at room temperature for 20 min and incubated at 37 °C in 5% CO_2 for 2–3 days to culture MNV-1 and for 7–8 days to culture HAV. The cells were then fixed with 2 ml of 3.7% formaldehyde for 4 h. The formaldehyde was discarded, and the agarose overlays were removed carefully with tap water. The fixed cells were stained with a 0.1% (w/v) crystal violet solution (Invitrogen; Carlsbad, CA., USA) for 20 min to visualize the plaques. Viral titers were calculated as the number of PFU per milliliter.