The antiviral action of common household disinfectants and antiseptics against murine hepatitis virus, a potential surrogate for SARS coronavirus

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Background: The 2003 outbreak of severe acute respiratory syndrome (SARS) infected over 8000 people and killed 774. Transmission of SARS occurred through direct and indirect contact and large droplet nuclei. The World Health Organization recommended the use of household disinfectants, which have not been previously tested against SARS coronavirus (SARS-CoV), to disinfect potentially contaminated environmental surfaces. There is a need for a surrogate test system given the limited availability of the SARS-CoV for testing and biosafety requirements necessary to safely handle it. In this study, the antiviral activity of standard household products was assayed against murine hepatitis virus (MHV), as a potential surrogate for SARS-CoV.

Methods: A surface test method, which involves drying an amount of virus on a surface and then applying the product for a specific contact time, was used to determine the virucidal activity. The virus titers and log reductions were determined by the Reed and Muench tissue culture infective dose $(TCID)_{50}$ end point method.

Results: When tested as directed, common household disinfectants or antiseptics, containing either 0.050% of triclosan, 0.12% of PCMX, 0.21% of sodium hypochlorite, 0.23% of pine oil, or 0.10% of a quaternary compound with 79% of ethanol, demonstrated a 3-log reduction or better against MHV without any virus recovered in a 30-second contact time.

Conclusion: Common household disinfectants and antiseptics were effective at inactivating MHV, a possible surrogate for SARS-CoV, from surfaces when used as directed. In an outbreak caused by novel agents, it is important to know the effectiveness of disinfectants and antiseptics to prevent or reduce the possibility of human-to-human transmission via surfaces.

Key Words: SARS; disinfectants; antiseptics; antiviral.

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The severe acute respiratory syndrome (SARS) outbreak infected 8098 people and killed 774 in 2003.[1] Because SARS was a new emerging disease, a medical treatment or vaccine was not available to prevent further cases or to help the infected survive. SARS coronavirus (SARS-CoV) was discovered in March of 2003 and shortly after was classified as a member of the family of viruses known as *Coronaviridae*.[2] However, recent phylogenetic analyses have suggested that SARS-CoV is equally related to any of the groups and belongs in a new group, group IV.[3,4] SARS-CoV and murine hepatitis virus (MHV) share many structural and genetic similarities. Both viruses have a similar genome organization and both contain 2 overlapping open reading frames (ORF) known as ORF 1a and ORF 1b, which are important to initiate translation.[5,6] SARS-CoV and MHV are enveloped viruses, and this structural similarity is very important when determining resistance and sensitivity to chemical disinfectants and antiseptics. One difference is that, whereas SARS-CoV is a biosafety level (BSL)-3 agent, MHV can be studied in a BSL-2 facility. Because SARS-CoV and MHV share many structural and genetic similarities and because MHV can be studied in a BSL-2 facility, the analysis of MHV may serve to answer questions about SARS-CoV more quickly and without the need to set up complex research facilities. MHV has the potential to be a suitable surrogate for SARS-CoV.

During the SARS outbreak, the recommendation of disinfectants and antiseptics to prevent transmission was one of the major steps for infection control. It has been documented that the SARS-CoV can survive up to 96 hours on surfaces, whereas other studies have shown the virus to retain its infectivity up to 6 days.[7] The effectiveness of disinfectants and antiseptics against the SARS-CoV was unknown. However, the lipophilic structure of SARS-CoV gave an indication that this virus, if similar to other lipophilic viruses, could easily be deactivated on surfaces from disinfectants. This would provide

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infection control for hospitals and households and help break the chain of transmission via surfaces.

The objective of this study was to assay the effectiveness of common household disinfectants and antiseptics against MHV, a virus from group II of the *Coronaviridae*, which in turn can be used as an indicator of how efficacious these disinfectants and antiseptics would perform against other members of the *Coronaviridae,* including SARS-CoV.

MATERIALS AND METHODS

Cell lines and viral stocks

The NCTC clone 1469 mouse liver cells (American Type Culture Collection [ATCC], Manassas, VA) were grown in Dulbecco's modified Eagle medium (ATCC, Manassas, VA) with 4 mmol/L L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose with the addition of 0.6 mL/L of gentamicin (Gibco Invitrogen Corporation, Carlsbad, CA) and 10% horse serum (ATCC, Manassas, VA) in an incubator at 37.0°C ± 2.5°C supplemented with 5% CO_2. Mouse hepatitis virus strain MHV-1 (Parkes) stocks (ATCC, Manassas, VA) were produced in a T75 flask containing the NCTC clone 1469 cells at >90% confluence and 2% horse serum. Virus stocks were stored at −75°C.

Virucidal test method

Multiwell plates (ie, 24-well assay plates) of mouse liver cells were seeded at least 1 day prior to the start of the assay procedure. On the day of the virucidal assay, an amount of 0.3 mL of virus stock was spread over a 28-cm^2 area marked on the underside of a hard, nonporous Petri dish and allowed to dry for 30 to 50 minutes at room temperature until a white, dry film was apparent.

A list of the products tested, the dilutions used during testing, and the yielding concentrations are outlined in Table 1. The products were chosen by the varying active ingredients. Those test substance samples that required diluting were prepared using sterile deionized water. The dilutions were performed based on manufacturer's instructions, except for the liquid hand soap, which was diluted further to reduce its viscosity. The contact time for all products was modified to 30 seconds. For each sample tested, 2 mL of test substance was applied directly to the virus film. Aerosolized products were used according to manufacturer's directions. At the 30-second contact time, 0.2 mL of the virus/test substance mixture was neutralized into 1.8 mL of media with 2% serum and serial 10-fold dilutions were performed. A total of 0.2 mL of each dilution was then inoculated into each of 4 wells containing the host cells. The assay plates were incubated at 37°C ± 2.5°C supplemented with 5% CO_2, and the cells were observed for toxicity or characteristic viral cytopathogenic effect at a minimum of over 7 nights by looking at the plates through an inverted microscope (Olympus Tokyo Inverted CK at 10× magnification, Olympus, Center Valley, PA) throughout the assay. The viral cytopathogenic effect was characterized by a degenerated cell sheet and cell detachment. To monitor the general health of the cells in each experiment, control wells containing cells grown in the absence of virus and product were observed throughout the course of the assay period. All tests were repeated 5 times with virus and toxicity controls.

Sephadex column preparation

A sephadex column was used to reduce test substance toxicity to the cell line for only some of the products. The sephadex slurry was prepared using a 1:20 dilution of lipophilic sephadex (Sigma-Aldrich Co., St. Louis, MO) in phosphate-buffered saline (Gibco Invitrogen Corporation). After an overnight incubation at 4°C, the slurry was then autoclaved for 20 to 25 minutes at 121°C/15 pounds per square inch (PSI) and allowed to cool to ambient temperature prior to use. Sephadex columns were prepared with the addition of 10 mL of sephadex slurry. Prior to the start of the virucidal assay procedure, the filled columns were centrifuged at 900 rpm in a CRU-5000 centrifuge for approximately 3 minutes. Once the column was prepared, 2 mL of the neutralized virus/test substance mixture in media with 2% serum was added to a sephadex column and centrifuged for 3 minutes at 900 rpm in a CRU-5000 centrifuge. The column flow-through was collected; this was used for inoculation into the 10^{-2} wells, and the rest of the method was followed as previously described. The columns were also used for all of the controls.

Calculation method

Infectious dose titers were determined by the Reed and Muench tissue culture infective dose $(TCID)_{50}$ end point method.[8,9] A minimum of 4 replicates were performed for each dilution. Several dilutions were used to quantify the virus titer and statistically determine the $TCID_{50}$ end point.[9] To express the titer in infectious units per unit volume, the reciprocal was taken and divided by the amount used for inoculation into the test wells. The end point of the test replicates was averaged between 2 replicates and then subtracted from the viral titer log recovery to determine the log reduction of each test substance.

RESULTS

Common household products were assayed against MHV, a proposed surrogate for SARS-CoV, to determine