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Short communication

A novel approach using an attenuated recombinant vaccinia virus to test the antipoxviral effects of handsoaps

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Abstract

Evidence indicates an increase in nosocomial and household infections due to viruses (Jeffries, D.J., 1995. Viral hazards to and from health care workers. J. Hosp. Infect. 30, 140–155). An antiviral assay was developed for evaluating efficiency of handsoaps at inactivating cell-free and cell-associated virus. A recombinant vaccinia virus, lacking a virulence factor (Isaacs, S.N., Kotwal, G.J., Moss, B., 1992. Vaccinia virus complement-control protein prevents antibody-dependent complement-enhanced neutralization of infectivity and contributes to virulence. Proc. Natl. Acad. Sci. USA 89, 628–632), whose construction was described earlier (Kotwal, G.J., Isaacs, S.N., McKenzie, R., Frank, M.M., Moss, B., 1990. Inhibition of the complement cascade by the major secretory protein of vaccinia virus. Science 250, 827–830), was used as the representative poxvirus. Two antibacterial handsoaps, one surgical handsoap, one moisturizing handsoap, and a sanitizing agent were tested. An aliquot of the virus was mixed and incubated with soap, then titrated onto BSC-1 cells for incubation at 37°C for 48 h. The soaps' effect on cell-associated virus was tested similarly. The antibacterial soaps inactivated all cell-free virus in 1 min. The surgical soap was effective with a 5-min incubation. None of the soaps eliminated all of the cell-associated virus in 1 min. This safe and reproducible assay seems efficient to establish the comparative efficacy of household and surgical soaps.

Keywords: Handwashing; Surgical hand disinfection; Antiviral testing; Vaccinia virus

Viruses are a leading cause of infectious diseases originating from nosocomial and household infections. Handwashing is the most effective means of stopping the spread of nosocomial infection (Marcil, 1993). In general, antibacterial handsoaps are often employed for this task. Consequently, it was decided to compare the relative antipoxviral effectiveness of these household products.

Although experimentation to test antiviral effects with household products such as Lysol has

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been conducted, Lysol is a disinfectant spray, not a handsoap (Ward et al., 1991). In a study using bovine rotavirus, it seems the most efficient method for removing the virus from finger tips was alcoholic solutions versus the much less effective soap and water and disinfectant detergents; however, a comparison of antibacterial handsoaps was not established (Bellamy et al., 1993). In an unrelated study, alcohol products tested in the presence of blood resulted in significantly greater reductions in numbers of colony-forming units than both a detergent containing 4% chlorhexidine gluconate (the active ingredient in the surgical soap tested, Hibiclens®) and a non-antimicrobial soap; yet this study did not contain information concerning viruses (Larson and Bobo, 1992). Alcoholic soap-free hand cleansers have recently been made available to the general public, but handsoaps are more widely used in practice. Thus, those individuals that consciously make the effort to use an antibacterial handsoap to combat 'germs' neglect the possibility that these handsoaps may offer no protection in daily encounters with viruses.

Poxviruses are once again gaining clinical importance due to their inclusion in the list of bioterrorism (e.g. small pox), as emerging viruses (e.g. monkey pox) and as opportunistic agents in AIDS (molluscum contagiosum). A recombinant vaccinia virus, vSIGK3, whose origin was described previously (Kotwal et al., 1990), was used as the infectious agent because this virus is safe to work with in the laboratory and is stable at room temperature, and therefore is a potential representative poxvirus for handsoap testing. Vaccinia is also ideal since it is a plaque-forming virus, which enables a rapid enumeration of viral titers.

Preliminary research with a 5-min incubation of 1×10^8 virus particles/ml of vSIGK3 and undiluted soap solution demonstrated that Dial® Antibacterial (Dial Corporation, Phoenix, AZ, USA), SoftSoap® Antibacterial (SSA; Colgate–Palmolive Company, New York, NY, USA), and a surgical soap, Hibiclens® (Zeneca Pharmaceuticals, Wilmington, DE, USA), effectively eliminated the virus. The active ingredient in Dial® and SSA is triclosan. Roccal II® 10% (Roccal) (National Laboratories Lehn & Fink Industrial Products Division of Sterile Drug Inc., Toledo, OH, USA), a sanitizing agent/germicide-algicide and deodorizer, was used as a comparison. Roccal's active ingredients are 12% Octylphenoxypolyethoxyethanol, 20% Alkyldimethylbenzylammonium chloride. These results suggested that all soaps were efficient at eliminating the infectivity of input virus in 5 min (data not shown). The rationale for choosing 1×10^8 particles/ml was that viruses such as hepatitis B may be present in titers of around 10^8 per ml of body fluid. Most other viruses do not exceed 10^6 particles per ml in body fluids (Lanphear, 1994).

In practice, very few people routinely wash their hands for 5 min. We therefore decided to test shorter reaction periods of 15, 30, and 60 s to estimate not only the effectiveness of the average person's handwashing (which could be shorter than the actual reaction periods), but also to determine the handsoaps' range of effectiveness. In addition, the handsoaps were diluted to a simulated working dilution of 1:20 with sterile water. Hibiclens® was not diluted since it is not diluted in normal use as a surgical soap. Another handsoap, SoftSoap® Moisturizing (SSM; Colgate–Palmolive Company, New York, NY, USA), was added to the study in order to observe the difference between it and SSA, and also because it functions as a negative control since it has no antimicrobial active ingredient.

The handsoaps were also tested using virus infected cells to determine their effects on cell-associated virus, simulating infectious blood or pus, since numerous viruses undoubtedly maintain their infectiousness on moist surfaces (Hilding, 1994). Soaps working efficiently in this manner would be an invaluable safety precaution in hospitals, daycares, schools, and homes, where body fluid-borne viruses are common. Studies have shown that blood remains in some areas of ungloved dental workers' hands, particularly subungual spaces, for at least 5 days — even with handwashing (Allen and Organ, 1982). Whether or not the remaining blood retains infectious properties after handwashing would be of importance to determine.

Pairs of six-well tissue culture plates (Falcon, Franklin Lakes, NJ, USA) containing confluent

monolayers of BSC-1 cells grown at 37°C were divided up into three, four-well sections. Section 1 received only virus, Section 2 received virus and handsoap, and Section 3 received only handsoap. Virus dilutions were added to a complete minimal essential medium (CMEM; Gibco BRL, Gaithesburg, MD, USA) containing 2.5% fetal bovine serum (FBS; Harlan, Indianapolis, IN, USA) with 1% penicillin, streptomycin, and fungizone.

All wells in Sections 1–3 had a total of 1 ml CMEM added, with virus and/or soap additions. For the initial dilution of 10^{-3} Section 1 1 μl vSIGK3 vaccinia virus (virus) was added to the 1 ml medium. This aliquot was then diluted to the 10^{-6}, 10^{-7}, and 10^{-8}. For use in Sections 2 and 3, the soaps first had to be brought to their average handwashing dilutions. The working dilution for the handsoaps was one part handsoap to 20 parts sterile water; Hibiclens® was not diluted since it is not diluted in normal use; Roccal was diluted for general disinfecting (one part cleaner to 1250 parts sterile water) as per manufacturer's instructions. Ten microliters of virus was added to 10 μl of any given handsoap solution, then incubated at room temperature for either 15, 30, or 60 s. The reaction period, or the amount of time that the virus alone is in contact with the working dilution of the given soap, ended when the mixture was added to the CMEM. For Section 2, the initial dilution of 10^{-2} was made by adding 20 μl virus + handsoap solution to 1 ml CMEM. This aliquot was then diluted down to 10^{-3}, 10^{-4}, and 10^{-5}. For the initial dilution of 10^{-2} of Section 3, 10 μl of the working dilution of a soap was added to 1 ml CMEM. This was then diluted down to 10^{-3}, 10^{-4}, and 10^{-5}.

In order to determine the titer of cell-associated virus, an additional plate of BSC-1 cells was grown at 37°C, but then infected at a multiplicity of 10 and harvested after 48 h, as described before (Joklik, 1962). The infected, scraped cells were centrifuged at $12\,500 \times g$. This pellet was suspended in 100 μl working dilution of the soap for a 60-s reaction period. Of this pellet + handsoap solution, 200 μl was added to 2 ml CMEM on the confluent monolayer to make the initial 10^{-2} dilution. This was then diluted down to 10^{-3}, 10^{-4}, and 10^{-5}. All plates, infected with either cell-free virus and cell-associated virus, were incubated at 37°C for 48 h, stained with crystal violet, and rinsed with water. Both sections of the experiment were conducted using Dial®, SSA, SSM, Hibiclens®, and Roccal.

To determine the number of surviving viral particles in the cell-free and intracellular viral solutions, the plaques from one well, visible after staining, were counted and then multiplied by the dilution factor. Plaque counts for Section 1 in the 60-s cell-free testing indicated that a consistent amount of virus was present in all trials; the average number of plaques was 1.5×10^{-8} per ml. None of the handsoap dilutions used adversely affected the cellular monolayer in the absence of virus. The raw data for cell-free and cell-associated virus was illustrated graphically (Fig. 1). Only the antibacterial soaps (Dial® and SSA) inactivated the cell-free virus in a 60-s reaction period. No significant inactivation was noted with the cell-associated virus in 60 s.

There was no greater than 2% difference between the titers obtained at different times with any given soap samples, suggesting a highly reproducible outcome when the protocol was strictly followed. It also suggested that there was no need for statistical analysis as long as the entire set of experiments was repeated at least twice. If the control virus used with a known titer did not come within $\pm 2\%$ of the expected titer, the experiment was repeated until the control virus was within $\pm 2\%$.

There have been no systematic studies reported on the antiviral effects of handsoaps using a human virus. This study, however, presents an effective assay, which may be used for further investigations. It should be emphasized that vaccinia cannot directly be compared to other viruses because it is extremely stable to both physical and chemical agents. Few other viruses can survive 0.5% phenol for 2–3 days. Therefore while vaccinia is a representative poxvirus, it is not a representative virus.

It is possible to further study the soaps and their reaction with vaccinia. More significantly, it is crucial to determine what needs to be done to make the soaps more effective, such as manufacturers altering the active ingredients or individuals

modifying their hygiene habits to allow for an adequate reaction period. In addition, it must be questioned as to why Hibiclens®, the surgical scrub, was less effective than the antibacterial handsoaps, Dial® and SSA. It may result from a difference in active ingredients: Dial® and SSA share the active ingredient triclosan, whereas Hibiclens®'s active ingredient is chlorhexidine gluconate. Perhaps the reduced efficacy of the surgical scrub's active ingredient is the reason why alcohol-based antiseptics, which appear to be a successful alternative in surgery preparation to detergent-based antiseptics, are being researched for use with shorter duration handwashing (Pereira et al., 1997). Further research using this assay could also include alcohol-based hand cleansers, which are currently on the market.

Many infections occur due to unknown and unexplained causes-prime examples are HCV infection, which currently infects 3.5 million people in the United States alone (Neiblum and Boynton, 1996), and the common cold. The common cold's modes of transmission and level of communicability are difficult to pinpoint (Jaakkola and Heinonen, 1995) for all strains and progress towards effective cold treatment in the past century has been scarce (Hilding, 1994).

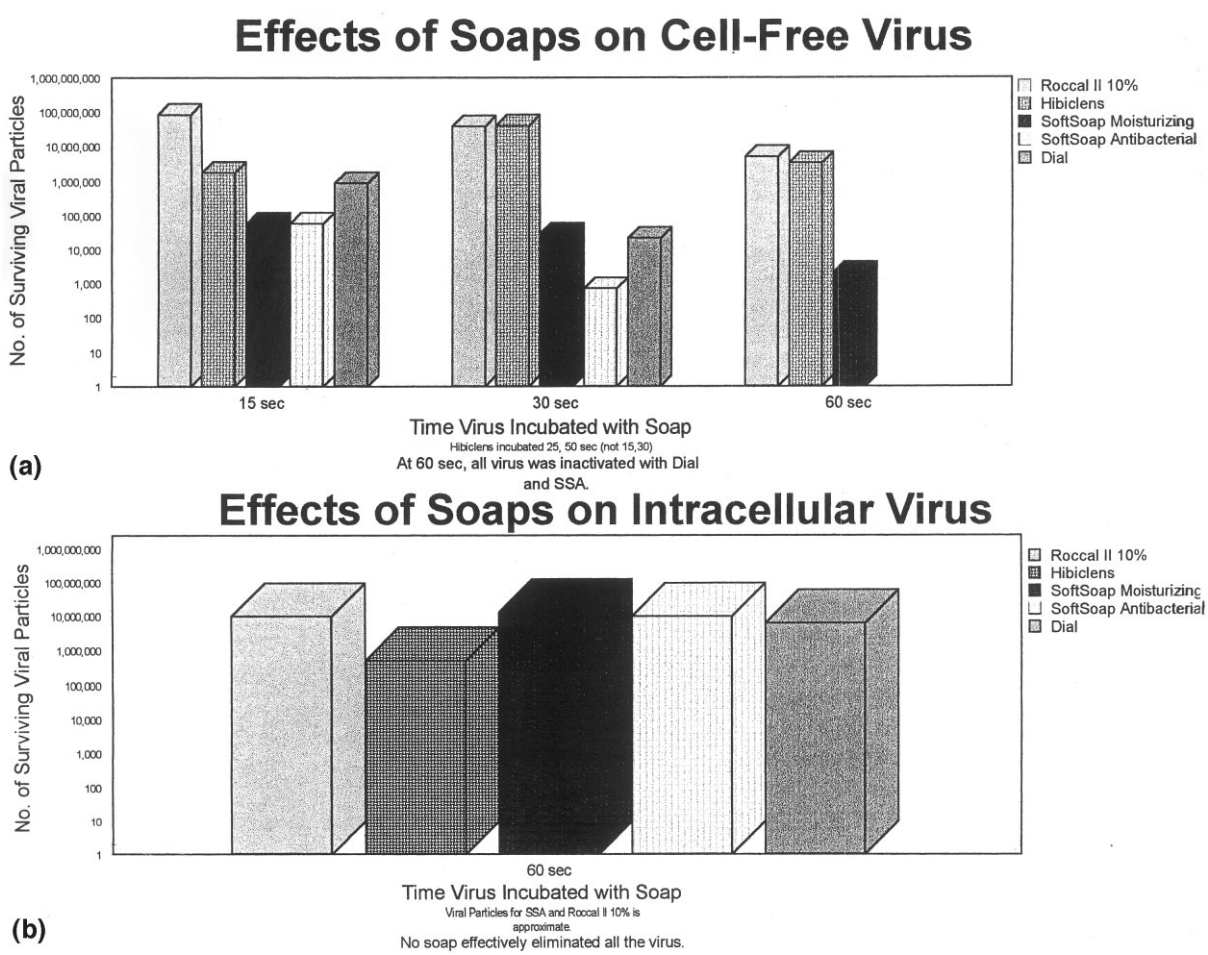

Fig. 1. a: Effects of soaps on cell-free virus. The graph indicates that as the reaction period increases, the number of surviving viral particles is reduced exponentially. At 60 s, all viral particles were inactivated by Dial® and SSA. b: Effects of soaps on intracellular virus. The graph indicates that the reaction period was not sufficient for any of the soaps to either eliminate or reduce the number of surviving viral particles. Note: the viral particle count for SSA and Roccal® was approximated.

Some researchers believe studies on a practical and efficient means to influence behavior in order to increase compliance with hand hygiene guidelines are needed more than elaborate and sophisticated studies on the effects of handwashing (Nystrom, 1994).

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