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Different Virucidal Activities of Hyperbranched Quaternary Ammonium Coatings on Poliovirus and Influenza Virus

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Virucidal activity of immobilized quaternary ammonium compounds (IQACs) coated onto glass and plastic surfaces was tested against enveloped influenza A (H1N1) virus and nonenveloped poliovirus Sabin1. The IQACs tested were virucidal against the influenza virus within 2 min, but no virucidal effect against poliovirus was found in 6 h.

Enteric and respiratory viruses can be transmitted directly from person to person but also indirectly via contaminated surfaces (1, 3, 4). Key factors in successful transmission are the quantity of infectious virus particles shed, the minimal infectious dose, the stability in the environment, and the resistance to disinfection (2). Consequently, cleaning and disinfection are means to control outbreaks and transmission of viruses; however, proper cleaning and disinfection can be laborious, and for nonenveloped enteric viruses such as noroviruses, the effectivity of currently practiced cleaning and disinfection procedures is not clear (5, 7). Therefore, self-decontaminating surfaces may be helpful in preventing transmission in health care settings, in food production areas, and in general from frequently touched and contaminated fomites. Applying antimicrobial coatings to produce self-decontaminating surfaces could help to interrupt the indirect transmission of pathogens and reduce the labor and time required for adequate cleaning and disinfection.

In this study, we coated plastic and glass surfaces with quaternary ammonium compounds (QACs). The immobilized QACs (IQACs) are comprised of hyperbranched polymers functionalized with tertiary amines, which are quaternized by alkylation with an alkylhalide. Various types of Hybrane hyperbranched polymers (HA1690, HA5290, DA17395, and DA33295, all from DSM, Heerlen, The Netherlands) were quaternized with heptylbromide ($C_7H_{15}Br$) to obtain various IQACs using the following procedure: a solution consisting of 10 g polymer, 7.1 g K_2CO_3, 70 ml *tert*-amylalcohol, and 12 ml heptylbromide was mixed and stirred for 24 h at 96°C. After the solids were removed by filtration, the polymer was precipitated by pouring the solution in a large excess of hexane or diethyl ether. After the polymer was washed with hexane or ether, the polymer was dried under vacuum. The polymer was dissolved in dry acetone, and the resulting solutions were used to form transparent films on glass slides (76 by 26 by 1 mm) and plastic wells (24-well plate; Corning, Germany) by applying a thin layer of fluid on the surfaces followed by drying under vacuum for 24 h. The polymers are very poorly water soluble and can be extracted only from the surface using organic solvents. These positively charged IQACs interact with the negatively charged outer surface of bacteria and change the permeability of the bacterial membrane. In this exploratory study, we determined the virucidal properties of several IQACs against the enveloped (lipophilic) respiratory influenza virus and nonenveloped (hydrophilic) enteric poliovirus.

Viruses used for the test were poliovirus Sabin1 (vaccine strain) and influenza A (H1N1) virus (clinical isolate, Hu/influenza A/266/2008/Netherlands [H1N1]). Virus stocks were prepared as described before (10). The poliovirus stock contained 1.6×10^7 50% tissue culture infective doses ($TCID_{50}$/ml), and the influenza A (H1N1) virus stock contained 4.0×10^6 $TCID_{50}$/ml. Fifty microliters of poliovirus or influenza virus were dispensed and spread at the center of each surface. Environmental persistence of both viruses was studied for 10 days on uncoated plastic and glass. The poliovirus was left on the coated surfaces for 1 and 6 h and influenza virus for 2 min and 1 h, respectively. The viruses were removed from the glass slides by swabbing with rayon swab sticks (Copan) as described before and from the plastic wells by rinsing with 1 ml cell culture medium (10). Visual inspection of the coatings after virus removal did not show any damage to the coat. Enumeration of the viruses and quantitative real-time reverse transcription (RT)-PCR was performed as described previously (10). Data for environmental resistance are presented as total infective viruses recovered from the surfaces (at $t = 0$ h, the suspension is not yet dried), and data for virucidal activity of the IQACs are presented as reduction relative to uncoated glass and plastic carriers. Recoveries of infective viruses after 1 h from the control carriers were 1.3% ± 0.0% and 45.0% ± 7.3% for influenza virus from glass and plastic, respectively, and 56.6% ± 9.2% and 89.7% ± 14.5% for poliovirus from glass and plastic, respectively. All experiments were performed at room temperature in duplicate, and the outcome is from two experiments. Statistical analysis was done using *t* test.

The persistence of poliovirus was high, with less than 1 $\log_{10}$ decay in 10 days (Fig. 1). Influenza virus showed fast decay the first 24 h, probably due mostly to drying, followed by slower decay up to day 5 and again faster decay after day 5 (Fig. 1). The decay in infective virus that could be retrieved from the coated carriers differed widely for poliovirus and influenza virus (Fig. 2). After 1 h, no significant reduction of infectious poliovirus was measured

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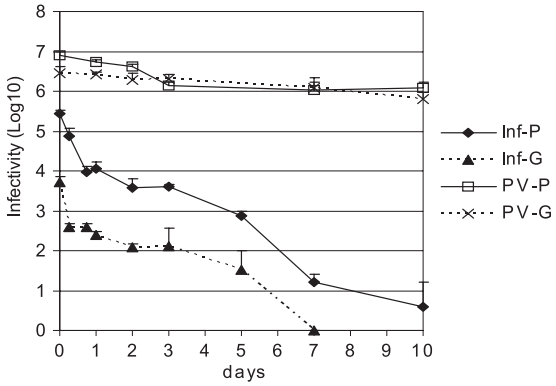

FIG 1 Environmental decay of poliovirus and influenza virus at room temperature. PV-P is poliovirus on plastic, PV-G is poliovirus on glass, Inf-P is influenza virus on plastic, and Inf-G is influenza virus on glass. Data are from 2 experiments with duplicate samples per experiment, and error bars indicate standard deviations (SD).

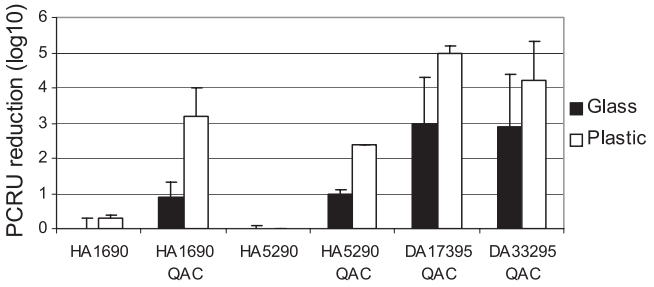

FIG 3 Reduction of PCRU of influenza virus by different IQACs after 1 h of exposure at room temperature. Data are from 2 experiments with duplicate samples per experiment, and error bars indicate SD.

for any of the coatings, while for influenza virus the reduction of infective virus was complete: no infective influenza viruses could be retrieved from the coated surfaces, except from the nonquaternized HA5290 polymer, for which no reduction was found. Identical levels of inactivation of influenza virus were already achieved after 2 min, while even after 6 h no significant reduction of poliovirus could be detected (data not shown). Remarkable was the complete inactivation of influenza virus by the quaternized and the nonquaternized HA1690 polymer. The reduction in infective influenza virus by quaternized HA1690 was concomitant with a significant reduction in PCR units (PCRU), while this was not true for the nonquaternized HA1690 (Fig. 3).

Even though quantitative data are missing, transmission of enteric and respiratory viruses via hard surfaces is a concern in health care settings, individual houses, and food production facilities. In this study, we found that no infective influenza viruses could be retrieved from the coated surfaces, indicating that the environmental survival of these enveloped respiratory viruses was reduced from over 5 and 10 days for glass and plastic, respectively, to less than 2 min on IQAC-coated surfaces. The virucidal mechanism of QACs has been described for the lipophilic enveloped viruses to involve disruption or detachment of the viral envelope, and enveloped viruses, such as herpes simplex virus, have been found to be very sensitive (8, 9). However, data on influenza viruses and QAC resistance are less unambiguous. In suspension tests, avian influenza H5N1 strains were not completely inactivated by 0.02% benzylalkonium chloride (manufacturers' recommended concentration) after 10 min (11), while in general QACs are considered good disinfection agents for influenza viruses (http://www.cdc.gov/h1n1flu/guidelines_labworkers.htm). We confirm virucidal effects of the IQACs tested against influenza viruses in our carrier tests. Further studies will be needed to determine how many times the coated surfaces can be cleaned and maintain this activity and if activity remains when surfaces are filthy or dusty.

The finding that no infective influenza viruses could be retrieved from the HA1690 base polymer-coated slides might be due to the length of the lipophilic tails and the high density of functional end groups (tertiary amines) which are available to be quaternized in the HA1690 base polymer (both tail length and density are higher for HA1690 than for HA5290). The full recovery of influenza virus RNA in the HA1690 base polymer shows that the infectivity reduction is not the result of a profound effect on the viral genome but indicates a primary effect on the virus envelope.

QACs have been reported to show low virucidal activity against hydrophilic nonenveloped viruses, such as picornaviruses. Infective poliovirus was reduced by 1.1 to 2.3 $\log_{10}$ by a QAC (alkyldimethylbenzyl ammonium chloride plus EDTA) in a suspension test (450 ppm, 10 min, 20°C) (6), while less than 1 $\log_{10}$ reduction was reported for a similar QAC in a comparable exposure in the presence of 20% blood but over 2.4 $\log_{10}$ in the absence of interfering substances (12). We report the lack of significant virucidal activity of the IQACs tested against poliovirus after up to 6 h of exposure. To the best of our knowledge, this is the first report on

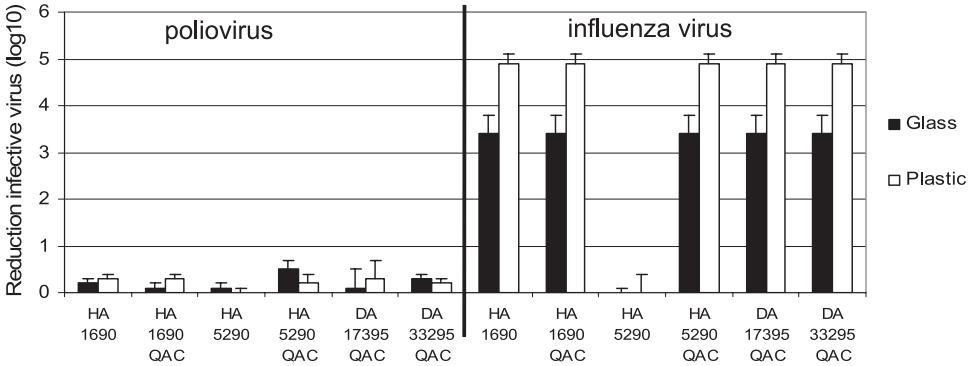

FIG 2 Reduction of infective poliovirus and influenza virus by different IQACs after 1 h of exposure at room temperature. Data are from 2 experiments with duplicate samples per experiment, and error bars indicate SD.

virucidal activity of IQACs against poliovirus and influenza virus, and we show that hyperbranched QAC coating is effective against influenza A (H1N1) virus but not effective against poliovirus Sabin1.

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