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Relationship of subtype influenza A pandemic strains to virucidal activity of a quaternary ammonium disinfectant

Daniel Prince, Herbert Prince, Chuan Wang

Gibraltar Laboratories Inc., Fairfield, NJ, USA.

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Introduction

Spread of influenza infection is mitigated not only through antiviral agents and vaccination, but also by treatment of environmental surfaces with surface disruptive chemical germicides. Little data is available on the comparative susceptibility of pandemic strains of Influenza A to chemical agents. We have embarked on a systematic study of the effect of various germicides on strains of influenza. The present work deals with BAC, a dual active antimicrobial agent accepted world-wide as both a disinfectant and antiseptic and various subtypes of Influenza A.[1] The major antigenic changes[2] in the influenza genome over the past 50 years have involved hemagglutinins H1, H2, and H3 producing the pandemics of 1957 (A/Japan/305/57/H2N2), 1968 (A/Hong Kong/8/68/H3N2), and the novel swine flu pandemic of 2009 (A/California/04/2009/H1N1). These are the subtypes that we have studied. Clearly, the question arises as to whether the changes in antigenicity are coupled with changes in germicide susceptibility.

Materials and methods

We have employed a modified log-reduction method[3] in a cell culture system employing MDCK cells[4] in serum-free Ex-Cell™[5] medium supplemented with trypsin. Microscopic examination of CPE was the marker for infectivity together with plaque assay. We confirmed antiviral potency by using specific subtype Influenza identification subtype technology, Quidel QuickVue® Influenza A + B Test.

Results

The log inactivation and percent inactivation by BAC after a 60 second contact time for the H1, H2, and H3 pandemic strains are as follows: A/Swine/Iowa/12/30 H1N1, 3·5 log/99·97%; A/Swine/Cal/2009 H1N1, 4·8 logs/99·998%; A/J305/57/H2N2, 5 logs/99·999%; and A/Hong Kong 8/68 H3N2, 5·0 logs/99·999% (Table 1).

Discussion

Comparable results of Antiviral efficacy are obtained with the $TCID_{50}$ and Plaque assays against all subtypes studied. When performing the plaque assay the sensitivity of virus recovery was better in the vessel with a larger surface area and overall recovery was in agreement with the potency determined by $TCID_{50}$ assay.

In our plaque assay, we inoculated A/Hong Kong/8/68 virus dilutions into two different vessels with 2 hours adsorption time: 6-well plate and T-25 flask, 9 ml inoculum per replicate. Virus titers obtained were: $1{\cdot}4 \times 10^6$ pfu/ml from 6-well plate and $2{\cdot}2 \times 10^6$ pfu/ml from T-25 flask (Table 2). The discrepancy on virus potency can possibly be explained as: the binding of virus to host cell occurs only when virus gets a chance to interact with the cell on the monolayer during adsorption time. The percentage of virus population in the inoculum that has the opportunity to bind to the cell mainly depends on the surface area where this interaction takes place. Therefore, in our experiment the plaque assay in the T-25 flask gave

Table 1. Efficacy of BAC against four strains of Influenza A using high titer viral pools

Antiviral = BAC 60 second contact time		H1N1 A/Swine/Iowa/15/30				H1N1 A/Swine/Cal/04/09				H2N2 A2/J305				H3N2 A2/Hong Kong/8/68				Representative inactivity all subtypes			
Viral Infectivity Assay	10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	T	T	T	T
	10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	T	T	T	T
	10^{-3}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
	10^{-4}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
	10^{-5}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
	10^{-6}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	0	0
	10^{-7}	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Cell control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
$TCID_{50}$		$10^{6.0}$				$10^{7.3}$				$10^{6.5}$				$10^{7.5}$				$\leq 10^{2.5}$			
Δ Log		≥3·5				≥4·8				≥4·0				≥5·0							

Legend: 0 = no virus; T = cytotoxic; + = virus

Table 2. Comparison of viral titer obtained in different vessels using quantal $TCID_{50}$ and plaque assay methods

Virus recovery			
Plaque assay		**$TCID_{50}$ assay**	
T-25 ($25\ cm^2$)	**6-well plate ($9\ cm^2$)**	**$TCID_{50}$/ml**	**TCID/ml**
$2{\cdot}2 \times 10^6$ pfu/ml	$1{\cdot}4 \times 10^6$ pfu/ml	5×10^6	$2{\cdot}5 \times 10^6$

higher virus recovery $2{\cdot}2\ 10^6$ versus $1{\cdot}4 \times 10^6$ pfu/ml. The increased virus recovery can translate into better sensitivity of the test system for disinfectant and antiviral agents. The potency of the virus used in this study was determined by $TCID_{50}$ was 5×10^6 $TCID_{50}$/ml.

Rapid diagnostic testing for influenza (QuickVue® Influenza A + B Test, Quidel) for AJ305 versus BAC was studied. The presence of Influenza viral nucleoprotein A determined by QuickVue kit correlated 100% with the viral infection based on by CPE in viral culture. Interestingly, the inactivation of viral nucleoprotein was able to be revealed with diagnostic kit in the dilutions of virus/BAC reaction mixture, which possessed prominent cytotoxic effect for the host cells in viral culture system. This type of molecular testing method is useful for interpreting antiviral efficacy against a background of cytotoxicity.

These experiments are intended for the sponsor to substantiate to US FDA that their antiviral substances are safe and effective. The data shows that the three hemagglutinin subtypes were highly susceptible to the Quaternary Ammonium Compound in the short term *in vitro* experiment. The appearance of novel subtypes in the future can be met with the assurance that disinfectant and/or antiseptic resistance will be unlikely. Certainly, from the above data, although genetic reassortment of human and swine viruses may modulate influenza pathogenesis and limit existing vaccine benefit, it is not likely be a factor in control of viruses on environmental surfaces by Benzalkonium-type disinfectant/cleaning agents in community or health care environments.

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Outbreak of viral respiratory infections in an aged care facility in NSW – lessons about a pandemic virus

Gulam Khandaker,[a] Bridget Doyle,[b] Dominic E. Dwyer,[c] Jiehui Yin,[a] Leon Heron,[a] Robert Booy[a]

[a]National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases, The Children's Hospital at Westmead and The University of Sydney, Sydney, NSW, Australia. [b]Public Health Unit, Greater Southern Area Health Service, Albury, NSW, Australia. [c]Centre for Infectious Diseases and Microbiology Laboratory Services (CIDMLS), Institute for Clinical Pathology and Medical Research (ICPMR), Westmead Hospital and The University of Sydney, Sydney, NSW, Australia.

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Abstract

Influenza in aged care facilities (ACFs) is associated with an increased risk of poor health outcomes among residents, including death. In this paper we share our experience of managing an outbreak of viral respiratory infection in an ACF very early in the 2009 influenza pandemic and also describe some of the emerging issues relating to cross-reacting antibodies to the pandemic (H1N1) 2009 influenza virus in the very elderly.

The outbreak investigation was conducted as part of an urgent public health intervention initiated by the New South Wales (NSW) Department of Health during the early stages of the first southern hemisphere wave of the 2009 pandemic. Nose and throat swabs for nucleic acid testing (NAT) plus acute and convalescent serum samples (6 weeks apart) were collected from all the residents of an ACF where an influenza-like illness (ILI) outbreak occurred.

The investigation revealed dual outbreaks of pandemic (H1N1) 2009 influenza and rhinovirus infection. Out of 28 residents, three had laboratory confirmed influenza [two with pandemic (H1N1) 2009], and 10 had rhinovirus infection on NAT. Testing of acute sera collected from every subject found elevated (≥1:40) pandemic (H1N1) 2009 HAI antibody in 60% (9/15) subjects aged 85 years or more (born before 1925 and median age 88 years; Geometric Mean Titre-GMT 48·1) compared with none of the 13 residents aged under 85 years (born after 1924 and median age 79 years; GMT 10·1, $P = 0{\cdot}01$). The ACF was closed to visitors for 7 days. The symptomatic residents received treatment-dose oseltamivir, and all other residents were given oseltamivir prophylaxis.

More than one virus may be circulating in an ACF with an ILI outbreak at any one time in winter. A significant proportion of elderly residents had pre-existing cross reacting antibody to the pandemic (H1N1) 2009, which may explain the minimal clinical impact of pandemic (H1N1) 2009 in this elderly population.

Introduction

Influenza is one of the leading causes of infectious death in elderly people, principally due to co-morbidities and declining immune competence with age. It is the most important agent in outbreaks of respiratory illness.[1] Influenza in aged care facilities (ACFs) is associated with an increased risk of poor health outcomes among residents, including death.[2] The clinical presentation of influenza in residents of ACFs can be subtle, with a blunted febrile response and a non-specific decline in mental and functional status.[3] Residents commonly have underlying diseases that can be exacerbated by influenza infection, and in addition, they are at higher risk of serious influenza-related complications than community dwelling elderly people.[4]

People aged over 65 years are also at higher risk of influenza-related death, and more than 90% of annual influenza-related mortality is usually confined to this high risk group.[5] In Australia, influenza and pneumonia have sub-

