Note

Evaluation of the Antiviral Activity of Chlorine Dioxide and Sodium Hypochlorite against Feline Calicivirus, Human Influenza Virus, Measles Virus, Canine Distemper Virus, Human Herpesvirus, Human Adenovirus, Canine Adenovirus and Canine Parvovirus

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We evaluated the antiviral activity of a chlorine dioxide gas solution (CD) and sodium hypochlorite (SH) against feline calicivirus, human influenza virus, measles virus, canine distemper virus, human herpesvirus, human adenovirus, canine adenovirus and canine parvovirus. CD at concentrations ranging from 1 to 100 ppm produced potent antiviral activity, inactivating ≥ 99.9% of the viruses with a 15 sec treatment for sensitization. The antiviral activity of CD was approximately 10 times higher than that of SH.

In recent years, the high incidence of norovirus infections in households, schools and nursing institutions for the elderly (Tsang et al., 2008) and the prevalence of measles in high schools (Sasaki et al., 2007) have become an increasing problem in Japan. In particular, norovirus can be transmitted orally, and it takes only a small amount of virus to cause acute gastroenteritis (Lindesmith et al., 2003) and sometimes even death among the elderly.

It is very important to develop safe and effective antiviral disinfectants. We evaluated the antiviral activity of a chlorine dioxide disinfectant, "chlorine dioxide gas solution" (long-life type, hereinafter referred

to as CD), and sodium hypochlorite (SH) against feline calicivirus (FCV, a surrogate for norovirus), human influenza virus (IFV), measles virus, canine distemper virus (CDV), human herpesvirus (HHV), human adenovirus (HAd), canine adenovirus (CAd) and canine parvovirus (CPV).

Effective antiviral disinfectants are needed to prevent infections of these highly contagious viruses. SH, a disinfectant commonly used against norovirus (Bruce et al., 1985), has the drawbacks of generating trihalomethane (Patterson et al., 1995; Wistrom et al., 1996), possessing an irritating odor, and undergoing a decrease in concentration during storage.

CD does not generate trihalomethane (Don, 1998), and the disinfecting effect of CD is less affected by pH compared to chlorine, has a less irritating odor

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and has a superior concentration stability during storage.

The viruses, strains and cells used in the present study were as follows: FCV: F4, CRFK; IFV: New Caledonia/20/99, Chick embryo; Measles virus: Edomonston, Vero/hSLAM; CDV: Onderstepoort, Vero; HHV-1: KOS, CRFK; HAd-2: wild, A549; CAd-2: Vaccine, BK; CPV: Y-l, CRFK.

The disinfectants studied were CD (Cleverin™, Taiko Pharmaceutical Co., Ltd., Osaka, Japan) and SH (Wako Pure Chemical Industries Ltd., Osaka, Japan).

All viruses except for IFV were prepared as follows. Cells cultured in Eagle's minimum essential medium (MEM) containing 10% fetal bovine serum (FBS), which was used as a growth medium, were inoculated with each virus and incubated at 37℃ until a cytopathic effect (CPE) was observed. The virus culture fluid was subjected to three freeze-thaw cycles and then purified and concentrated. Regarding IFV, after the virus culture fluid was inoculated into the chorioallantoic cavity of 10-day-old embryonated chicken eggs and incubated for 3 days at 34℃, the chorioallantoic fluid was harvested.

Virus culture fluids were subjected to low-speed centrifuge for cell removal, and then concentrated and purified through a 20% sucrose cushion. To the resultant sediment, 2 mL of distilled water was added, and the virus culture was ultimately concentrated to 500 times its original concentration. Virus suspensions were sterile-filtered through a 0.45 μm-pore size membrane filter, and the filtrate was stored frozen at -80℃ until ready for use.

Drug treatment of viruses was performed as follows. Each drug was diluted with sterilized distilled water for concentration adjustment. To 240 μL of each drug at each concentration（1,250 ppm, 125 ppm, 12.5 ppm, 1.25 ppm, 0.125 ppm and 0.0125 ppm）, 60 μL of virus filtrate was added and stirred for 15, 30, 60, 120 and 180 seconds for sensitization. Then, from each reaction solution, 50 μL of virus was harvested, and 450 μL of 5 mM sodium thiosulfate was added to neutralize the disinfectant. As virus controls, viruses treated with distilled water instead of diluted drugs were used. Tenfold serial dilutions of neutralized viruses were prepared with MEM to determine the infectivity titer (50% tissue culture infective dose [TCID$_{50}$]). The concentration of SH and CD were estimated by the DPK kit (type CN-66F, HACH Com. Iowa, USA) and spectrophotometric method (Sjestrem and Disa, 1976), respectively. All examinations were carried out in biosafety level 2 laboratories.

Infectivity titer determination for FCV, IFV, measles virus, CDV, HHV-1, HAd-2, CAd-2 and CPV was carried out as follows. Host cells for each virus plated in a 96-well microplate（100 μL/well）were incubated for 3 days at 37℃ in a 5% carbon dioxide incubator. The cells were then washed with MEM, and inoculated with the diluted virus（4 wells for each dilution, 50 μL/well）. After 60 min of viral adsorption onto the cells at 37℃ in a 5% carbon dioxide incubator, the cells were washed with MEM twice. After incubation for 5 days at 37℃ with an addition of 100 μL of MEM in a 5% carbon dioxide incubator, TCID$_{50}$/50 μL was calculated by microscopic examination of CPE. Antiviral activity（%）was calculated as follows: antiviral activity（%）＝{1－(infectivity of each treated virus/virus control)}×100

Table 1 shows the reduction of infectivity of various viruses by the CD and SH treatment.

Against FCV, CD exhibited no measurable effect with 1.0 ppm treatment for 180 sec, but produced antiviral activity against ≧ 99.99% of the virus with 10 ppm treatment for 15 sec. SH had no antiviral effect at 10 ppm, but showed antiviral activity against ≧ 99.99% of the virus at 100 ppm treated for 15 sec.

Against IFV, CD produced antiviral activity against ≧ 99.99% of the virus with 1.0 ppm treatment for 15 sec. On the other hand, antiviral activity against 99.99% of the virus was obtained for SH with 100 ppm treatment for 15 sec.

Against Measles virus, antiviral activity against ≧ 99.99% of the virus was observed at 10 ppm for 30 sec or at 100 ppm for 15 sec with CD treatment, and at 100 ppm for 30 sec or at 1000 ppm for 15 sec with SH treatment.

Against CDV, antiviral activity for 99.99% of the virus was demonstrated at 10 ppm for 15 sec for CD treatment and at 100 ppm for 30 sec with SH treatment.

Against HHV-1: CD produced antiviral activity against 99.9% of the virus with 10 ppm treatment for 15 sec, while SH treatment at 10 ppm for 180 sec resulted in antiviral activity against 99% of the virus.

Against HAd-2 and CAd-2, antiviral activity of CD was observed against 99.99% of the virus with 10 ppm treatment for 15 sec, whereas SH required a concentration ten times higher than that of CD to produce an equivalent effect.

However CPV was resistant to both disinfectants; antiviral activity against ≧ 99.99% of this virus was obtained at 10 ppm for 180 sec or at 100 ppm for 15 sec with CD treatment in comparison to SH treatment at 100 ppm for 120 sec.

The present study examined the antiviral activity of CD and SH against RNA viruses such as FCV, IFV, measles virus and CDV, as well as DNA viruses such

TABLE 1. Reduction of infectivity of various viruses by the treatment with CD and SH

Virus	Disinfectants	Concentration ppm	Virus control	Treatment (seconds)				
				15	30	60	120	180
FCV	Chlorine dioxide	0.1	6.75	6.75	6.75	6.75	6.75	6.75
		1.0	6.75	6.75	6.75	6.75	6.75	6.5
		10	6.75	1.5	1.0	1.0	0.75	0.75
		100	6.75	<0.5	<0.5	<0.5	<0.5	<0.5
	Sodium hypochlorite	1	6.75	6.75	6.75	6.5	6.5	6.5
		10	6.75	6.5	6.5	6.5	6.25	6.25
		100	6.75	0.75	0.75	0.75	0.75	0.75
		1000	6.75	<0.5	<0.5	<0.5	<0.5	<0.5
IFV	Chlorine dioxide	0.1	6.5	4.75	4.5	4.0	3.75	3.75
		1.0	6.5	2.25	2.25	2.0	1.5	1.5
		10	6.5	<0.5	<0.5	<0.5	<0.5	<0.5
		100	6.5	<0.5	<0.5	<0.5	<0.5	<0.5
	Sodium hypochlorite	1	6.5	5.5	5.5	5.5	5.5	4.75
		10	6.5	3.5	3.5	3.5	2.75	2.75
		100	6.5	<0.5	<0.5	<0.5	<0.5	<0.5
Measles virus	Chlorine dioxide	1.0	5.5	4.5	4.25	4.0	4.0	3.75
		10	5.5	2.0	<0.5	<0.5	<0.5	<0.5
		100	5.5	<0.5	<0.5	<0.5	<0.5	<0.5
	Sodium hypochlorite	1.0	5.5	5.0	4.75	4.75	4.75	4.75
		10	5.5	4.5	4.5	3.75	3.75	3.75
		100	5.5	4.75	<0.5	<0.5	<0.5	<0.5
		1000	5.5	<0.5	<0.5	<0.5	<0.5	<0.5
CDV	Chlorine dioxide	0.1	5.5	5.5	5.5	5.5	5.5	5.5
		1.0	5.5	2.75	1.75	1.75	1.75	1.75
		10	5.5	<0.5	<0.5	<0.5	<0.5	<0.5
		100	5.5	<0.5	<0.5	<0.5	<0.5	<0.5
	Sodium hypochlorite	1.0	5.5	5.5	5.5	5.5	5.5	5.5
		10	5.5	5.5	5.5	5.5	5.5	5.5
		100	5.5	2.5	1.5	<0.5	<0.5	<0.5
		1000	5.5	<0.5	<0.5	<0.5	<0.5	<0.5
HHV-1	Chlorine dioxide	0.1	5.0	4.25	4.0	3.75	3.75	3.75
		1.0	5.0	3.75	3.50	3.0	3.0	3.0
		10	5.0	1.5	1.25	1.25	1.25	1.25
	Sodium hypochlorite	0.1	5.0	4.5	4.25	4.25	4.25	4.25
		1.0	5.0	4.75	4.5	4.25	4.25	4.25
		10	5.0	3.75	3.75	3.25	3.0	3.0
HAd-2	Chlorine dioxide	0.1	6.5	6.0	6.0	6.0	6.0	6.0
		1.0	6.5	5.0	5.0	5.0	5.0	5.0
		10	6.5	1.5	1.5	1.5	1.5	1.5
		100	6.5	<0.5	<0.5	<0.5	<0.5	<0.5
	Sodium hypochlorite	1.0	6.5	6.0	6.0	6.0	6.0	6.0
		10	6.5	5.5	5.5	5.5	5.5	5.5
		100	6.5	1.5	1.5	1.5	1.5	1.5
		1000	6.5	<0.5	<0.5	<0.5	<0.5	<0.5
CAd-2	Chlorine dioxide	0.1	6.5	6.0	6.0	6.0	6.0	6.0
		1.0	6.5	6.0	6.0	6.0	6.0	6.0
		10	6.5	<0.5	<0.5	<0.5	<0.5	<0.5
		100	6.5	<0.5	<0.5	<0.5	<0.5	<0.5
	Sodium hypochlorite	1.0	6.5	6.0	6.0	6.0	6.0	6.0
		10	6.5	6.0	6.0	6.0	6.0	6.0
		100	6.5	<0.5	<0.5	<0.5	<0.5	<0.5
		1000	6.5	<0.5	<0.5	<0.5	<0.5	<0.5
CPV	Chlorine dioxide	0.1	4.5	4.25	4.5	4.5	4.5	4.5
		1.0	4.5	4.5	4.5	4.5	4.5	4.5
		10	4.5	3.25	2.25	2.0	1.0	<0.5
		100	4.5	<0.5	<0.5	<0.5	<0.5	<0.5
	Sodium hypochlorite	0.1	4.5	4.5	4.5	4.25	4.25	3.75
		1.0	4.5	4.0	4.0	4.0	4.5	4.5
		10	4.5	2.75	2.75	2.25	2.0	2.0
		100	4.5	2.75	2.5	1.0	<0.5	<0.5

Each value represents the $\log_{10}$ TCID$_{50}$/50 μL of virus determination

as HHV, HAd-2, CAd-2 and CPV.

Antiviral activity against norovirus was determined using FCV. CD at 10 ppm for 15 sec inactivated ≥ 99.99% of the virus, whereas SH required 10 times higher concentrations than CD to produce an equivalent effect. This observation indicates that CD has the potential to be used as a disinfectant against norovirus.

In addition, against other viruses, CD was equivalent to SH in terms of its antiviral activity at 1/10 concentrations.

Treatment with these disinfectants for sensitization at lower concentrations for up to 180 sec was performed; longer durations of treatment were not associated with any increase in antiviral activity. Meanwhile, sensitization at higher concentrations resulted in a more potent antiviral effect as indicated by the infectivity titer, showing that the disinfectant concentration is an important factor for virus inactivation.

CD exhibited antiviral activity in the concentration range from 0.1 to 10 ppm, which was 1/10 that of SH. Having antiviral activity at lower concentrations than SH, CD is expected to be useful as a virus disinfectant.

CD, which has demonstrated antiviral activity and is known to be less likely to generate trihalomethane (Wistrom et al., 1996), is a potent alternative disinfectant to SH (Junli et al., 1997). The CD used in the present study has the advantage of maintaining concentration levels of chlorine dioxide over an extended period. Since chlorine dioxide is also available in gaseous form (Ogata and Shibata., 2008), it can be directly sprayed in wards and laboratories in hospitals, meal preparation rooms in school lunch centers and other places, which may contribute to better prevention of hospital-acquired infections and food poisoning.

In the present study, the antiviral activity of disinfectants was determined under contamination-free conditions. Since it has been reported that contamination of disinfectants with proteins or other foreign materials results in decreased antiviral activity, it will be necessary to assess the antiviral activity using purified virus solutions containing FBS or other appropriate contaminants.

In Europe and the US, the use of chlorine dioxide to disinfect tap water has been implemented (Aleta and Berg, 1986; Lykins et al., 1990). The results of the present study provide an additional support for the use of CD as an antiviral agent; it also has several advantages over SH. Therefore, CD may be used more frequently in the future.

While detailed reports on the mechanisms of SH's antiviral activity are not available to our knowledge, CD has been reported to produce its antiviral activity by acting on virus nucleic acids and on virus proteins (Li et al., 2002), and by oxidizing amino acids tryptophan and tyrosine (Ogata and Shibata, 2008). In the present study, CD's antiviral activity was observed against both RNA and DNA viruses regardless of the presence/absence of the envelope, which is interesting to clarify the mechanisms of action. The higher antiviral activity of CD compared to that of SH may be attributable to differences in the mechanisms of action.

To confirm the feasibility of CD use in clinical and environmental settings, assessment of the viral growth inhibitory effect of CD under conditions of protein contamination and of the use of CD in aerosol form is necessary.

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