Virucidal effect of disinfectants on several animal viruses

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Abstract

Nine disinfectants were tested at usual concentrations for virucidal efficiency on 4 DNA and 9 RNA viruses by means of the Sephadex LH-20 gel filtration method. Phenol group reagents showed perfect virucidal activity on all of the DNA and RNA viruses whether enveloped or uneveloped. Alcohol group and surface active agents had virucidal effect on several enveloped viruses. In incorporation of virus and yeast at organic substance, the virucidal effect of surface active agent was completely suppressed, and a partial decrease of the effect of halogen compound was also observed on each virus tested.

Key words : Disinfectants, Viruses

Introduction

Disinfectants are used for expectative destructible effects on contaminating bacterial and viral pathogens in many research laboratories. Bactericidal mechanisms of disinfectants are relatively well known ; degeneration of cellular protein in bacteria treated with alcohol and aldehyde groups, and in the treatment of phenol and halogen groups, coagulation of cellular protein in high concentration and inactivation of bacterial enzymes in low concentration. In Japan the comparable estimation of bactericidal disinfectants has been carried out according to the standard manuals which are provided by the Ministry of Health and Welfare. Such mechanisms have not yet been fully explored on virucidal disinfectants. There is a report on the destruction of viral RNA in chlorine inactivation of polioviruses ; no effect of chlorine on the viral capsid protein was observed[4]. In future virucidal activity of disinfectants will have to be compared with the standard method established by an adequate organization. The present paper, however, is concerned with virucidal efficacy of 9 disinfectants against 13 viruses through the Sephadex LH-20 gel filtration method[2].

Materials and Methods

Disinfectants

The 9 disinfectants used are as follows : 2 alcohols (ethanol, Amakas Chemical Co., Ltd., Tokyo, isopropanol, Kanto Chemical Co., INC, Tokyo), formaldehyde (Kanto Chemical Co., INC, Tokyo), 2 phenolitics (phenol, Kanto Chemical Co., INC,

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Tokyo, saponated cresol, Kenei Pharm. Co., Ltd., Osaka), 2 halogen compounds (iodophor, Pfizer Taito Co., Ltd., Tokyo, sodium hypochlorite, Kanto Chemical Co., INC, Tokyo) and 2 surface active agents (benzalkonium chloride, Kenei Pharm. Co., Ltd., Osaka, didecyldimethyl ammonium chloride, Sumitomo Pharm. Co., Ltd., Osaka).

Viruses and cell cultures

The HH-1 strain of equine rhinopneumonitis virus (ERV), the C7301 strain of feline rhinotracheitis virus (FRV), the Woc-4 strain of infectious canine hepatitis virus (ICHV) and the Abashiri strain of mink enteritis virus (MEV) were used as DNA viruses, and as RNA viruses, the HEP-Flury strain of rabies virus (RBV), the MI-110 strain of Getah virus (GTV), the Sato strain of Newcastle disease virus (NDV), the SN-48 strain of avian infectious bronchitis virus (IBV), the JaGAr#01 strain of Japanese encephalitis virus (JEV), the SA-11 strain of simian rotavirus (SRV), the Lincoln strain of Nebraska calf diarrhea virus (NCDV), the No. 1 strain of feline calicivirus (FCV), and the PV-1 strain of infectious bursal disease virus (IBDV). These viruses were passaged and prepared in appropriate tissue culture systems or chick embryonated eggs (IBV), ERV in RK-13 cells, ICHV in MDCK cells, MEV, FRV and RCV in CRFK (cat kidney) cells, RBV and GTV in BHK-21 cells, NDV and IBDV in primary chick embryo fibroblast cells, JEV in C6/36 cells, SRV and NCDV in MA 104 cells, respectively. These cells were cultured in Eagle's minimum essential medium (Nissui Seiyaku Co., Ltd., Tokyo) supplemented with antibiotics and calf or fetal calf serum. The same medium without serum was used as maintenance medium containing 0.5 μg/ml trypsin (bovine origin, type 3, Sigma Chemical Co., Ltd., USA) for the cells after inoculation of SRV or NCDV. The virus titers of stock solution and the virus titers after chemical inactivation were titrated with 10-fold dilution method in each of culture cells or chick embryonated eggs. The titers were expressed as 50% tissue culture or egg infectious dose/ml with cytopathic effects in RFV, ICHV, FRV, GTV, JEV, SRV, NCDV and FCV, with typical intranuclear inclusions in MEV, and with hemorrhagic death of chick embryos in IBV, and as plaque-forming unit/ml in RBV, NDV and IBDV.

Assay procedure

Chemical inactivation of viruses and detoxification of the virus-disinfectant mixtures were performed with essentially the same method described by Scott[5] and Blackwell and Chen[1]. Briefly, 1 ml of stock virus solution was combined with the same volume of each disinfectant, except ethanol, in an adequate concentration. In the ethanol 1 ml of the virus was mixed with 2.33 ml of ethanol. Mixtures were allowed to react for 10 min in water bath at 20°C then immediately detoxified by gel filtration method of which a double centrifuge tube consisting of inner 15 ml of round-bottom tube which was placed a small hole in the bottom and filled with Sephadex LH-20 (Pharmacia Fine Chemicals AB Uppsala, Sweden), and of outer 50 ml of V-bottom tube[11].

Results

As shown in Table 1, 4 enveloped viruses, ERV, FRV, RBV and GTV were inactivated completely by 70% ethanol, 50% isopropanol, 3% phenol and 1% saponated cresol. Moreover, ERV, FRV and GTV were inactivated by 200 ppm sodium hypochlorite, and ERV and GTV, by 52 ppm iodophor, respectively. Unenveloped viruses, ICHV, SRV, and FCV, were inactivated completely by 3% phenol and 1% saponated cresol. SRV also was inactivated by various reagents such as 70% ethanol, 50% isopropanol, 200 ppm sodium hypochlorite and 10 ppm iodophor. Complete inactivation of DNA viruses and the moderate effect to RNA viruses were observed by the treatment of 2% formalin. Surface active agents, benzalkonium chloride and didecyldimethyl ammonium chloride, were effective against only enveloped viruses among the 3 DNA and 8 RNA viruses tested at a concentration of 0.05% (Table 2).

Table 1. Virucidal activity of disinfectants against seven viruses

Disinfectant	Final concentration	DNA viruses			RNA viruses			
		ERV (5.8)[a]	FRV (5.6)	ICHV (6.5)	RBV (7.1)	GTV (5.8)	SRV (5.1)	FCV (7.1)
Ethanol	70%	+−+[b]	+++	±	+++	+−+	+++	+ +
Isopropanol	50%	+++	+++	+	+−+	+−+	+++	−
Formalin	0.5%	•[c]	+++	•	±	•	+	•
	2.0%	+−+	+++	+−+	+ +	+ +	+ +	+
Phenol	3.0%	+−−	+++	+−+	+++	+++	−++	+++
Saponated cresol	1.0%	+−−	+++	+−+	+++	+++	−++	+++
Sodium hypochlorite	10ppm	•	−	•	−	•	−	•
	200ppm	+++	+−+	±	+	−++	+++	±
Iodophor	5ppm	•	±	•	−	•	+ +	•
	10ppm	•	±	•	−	•	+++	•
	52ppm	+−+	•	±	•	+++	•	+ +

[a] Logs of virus titers before inactivation.
[b] Activity rating of disinfectants in Log_{10} virus inactivated; − : $<1.0log_{10}$ ($\leq 10\%$), ± : $1.0-1.9log_{10}$ (10−99%), + : $2.0-2.9log_{10}$ (99.0−99.9%), + + : $3.0-3.9log_{10}$ (99.0−99.9%), + + + : $\geq 4.0log_{10}$ ($\geq 99.99\%$).
[c] Not tested.

Table 2. Virucidal activity of surface active agents against several viruses

Agent	Final concentration	DNA viruses			RNA viruses						
		ERV (7.8)[a]	ICHV (6.5)	MEV (4.5)	RBV (7.1)	GTV (5.9)	NDV (6.3)	IBV (5.2)	JEV (5.7)	NCDV (7.6)	FCV (6.8)	IBDV (4.6)
BZC[b]	0.05%	+++[c]	+	−	+++	++−	+++	+++	+++	++	±	±
DAC	0.05%	+−+	+	±	+++	++−	+++	+++	+++	++	−	+

[a] Logs of virus titers before inactivation.
[b] BZC: Benzalkonium choride DAC: Didecyldimethyl ammonium chloride.
[c] See Table 1 for key.

Table 3. Effect of 5% yeast on virucidal activity of disinfectants

Disinfectant	Final concentration	ERV		ICHV		FCV		GTV	
		None (5.8)[a]	Yeast (4.9)	None (6.5)	Yeast (6.5)	None (7.4)	Yeast (7.1)	None (5.3)	Yeast (5.8)
Ethanol	70%	+++[b]	+++	•[c]	•	•	•	+++	−++
Isopropanol	50%	+++	++−	•	•	•	•	−++	+++
Formalin	2.0%	+++	+++	+++	+−+	+	+ +	+ +	+
Phenol	3.0%	+++	++−	+++	+++	+++	+++	−++	−++
Saponated cresol	1.0%	+++	+++	+−+	+++	−++	−++	+++	+++
Benzalkonium chlo.	0.05%	++−	±	•	•	•	•	+++	+++
Sodium hypochlorite	200ppm	+++	+−+	•	•	•	•	+++	++−
Iodophor	52ppm	+++	+−+	±	−	+ +	+	+++	+

[a] Logs of virus titers before inactivation.
[b] See Table 1 for key.
[c] Not tested.

The incorporation effect of 5% yeast as organic substance on virucidal activity of the same reagents as described above was examined on ERV and GTV (Table 3). The results indicated that the virucidal effect of 0.05% benzalkonium chloride on ERV was completely suppressed, and a partial decrease (99.94%) of the effect of 52 ppm iodophor was observed on GTV.

Discussion

Dilution[2] and reduction[8] methods are well known for the measuring of virucidal activity of various disinfectants. In the former method, however, there are some difficulties about the low infectious dose of test viruses and the cytotoxicity of low dilutions of mixtures of virus and reagent against culture cells. Some cases in the latter method lack a suitable agent to reduce the activity of disinfectants used. In the so-called sandwich method, a specific method reported by Spillmann et al[6] and Traub et al[7], the test virus was adsorbed onto an electropositive membrane filter which was then sandwiched between 2 polycarbonate membranes with pores smaller than the virus diameter. After exposure to a disinfectant, the surviving fraction of virus was eluted from the inner filter and determined by a suitable virus titration. In the sandwich method or in Sephadex LH-20 gel filtration, the virus titers following chemical treatment were determined exactly in culture cells. This was because a cytotoxic substance from the mixtures of virus and reagent may be completely removed in both methods.

It is known that the enveloped viruses were more sensitive to chemical disinfectants as compared with the unenveloped viruses. In the present study also, the enveloped viruses tested were inactivated completely by the treatment of many disinfectants such as ethanol, isopropanol, benzalkonium chloride and didecyldimethyl ammonium chloride. However, no effect of these reagents on any of the unenveloped viruses except SRV was recognized.

Phenol and saponated cresol had a wide range of virucidal effects on viruses tested in the present study. The same findings were recently obtained on Sendai virus, canine distemper virus, lymphocytic choriomeningitis virus, murine hepatitis virus, canine coronavirus and hemorrhagic fever with renal syndrome virus in the same gel filtration method[3,9]. In general text books, however, phenol and cresol reagents were reported to have no disinfectant effect on many viruses. Further literature dealing with the virucidal experiment of these reagents was scanty. This discrepancy is due to the very little available information on the virucidal effect of these reagents because of the development of severe cytotoxicity to the culture cells in dilution and reduction methods. Although the reagents were not influenced by yeast used as organic substance, they give off a bad odor and have stimulative action on the mucosa or the derma of human beings and animals. Therefore it seems that these reagents may be limited in their virucidal use by their side-effects.

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各種動物ウイルスに対する消毒剤の殺滅効果

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摘　要

消毒剤9種類のDNAウイルス4種類とRNAウイルス9種類に対する殺滅効果を，Sephadex LH-20ゲル濾過法により，常用濃度で比較検討した。その結果，アルコール類と界面活性剤はエンベロープを有するウイルスに対し極めて有効であり，フェノール類はエンベロープの有無に関係なく供試したDNAおよびRNAウイルスの全てに有効であった。また，有機物としてイーストを5%に添加した時，界面活性剤では完全に，ハロゲン化合物では部分的に，ウイルス殺滅効果がそれぞれ抑制された。

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