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SHORT COMMUNICATION

Development of a new disinfectant with very strong anti-influenza viral activity: a preliminary report

Hiroki Takakuwa · Toshiyuki Maruoka · Tadayo Hata · Masaaki Miyazawa · Tomoyo Hata · Hitoshi Toshimori · Koichi Otsuki

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Abstract
Objectives We evaluated the effectiveness and safety of a disinfectant newly developed by our laboratories for use against influenza viruses.
Methods The effectiveness of our new disinfectant against avian, swine and human influenza viruses was tested in ovo. The acute toxicity of this disinfectant to two different cultured cell lines was investigated.
Results This new disinfectant showed very strong anti-influenza viral activity in the in ovo tests. All of the influenza viruses tested were inactivated very quickly. Following exposure to the disinfectant, the infectivity of all viral strains tested had been eliminated within $\leq$10 min. The infectant showed a weak acute toxicity in vitro.
Conclusion This new disinfectant is expected to be useful for preventing viral infection during a new influenza pandemic.

H. Takakuwa · K. Otsuki (✉)
Avian Influenza Research Centre,
Kyoto Sangyo University,
Motoyama Kamigamo, Kita-ku,
Kyoto 603-8555, Japan
e-mail: k3666@cc.kyoto-su.ac.jp

T. Maruoka · Tadayo Hata · Tomoyo Hata · H. Toshimori
Bacteria Research Center,
Shionomiya Hot Spring Hospital–Zaidanhojin Seikenkai,
Tondabayashi, Osaka, Japan

M. Miyazawa
Department of Immunology,
Kinki University School of Medicine,
Osakasayama, Osaka, Japan

K. Otsuki
Avian Zoonoses Research Centre,
Faculty of Agriculture, Tottori University, Tottori, Japan

Keywords Avian influenza virus · Human influenza virus · New disinfectant · Strong activity · Swine influenza virus

Introduction

Outbreaks of highly pathogenic avian influenza and other emerging and re-emerging diseases have caused serious economical and social disturbances worldwide [1–4]. Although the pandemic H1N1 subtype influenza virus has rapidly spread throughout the world since the end of April 2009, the production of a new influenza vaccine is still insufficient. However, the preparation of large amounts of medicine effective against influenza was also difficult prior to the occurrence of this latest pandemic. Therefore, there is a need to develop possible control methods, such as an easily obtained, effective disinfectant to prevent the virus from spreading. Our laboratories have succeeded in developing a new disinfectant which consists mainly of an iron ion. Tests have demonstrated that this disinfectant in very efficient in rapidly inactivating bacteria and influenza viruses.

Materials and methods

Experiment 1

The new disinfectant was prepared as follows. First, solution A was made by dissolving 0.96 g $FeCl_3 6H_2O$ in 200 ml distilled water. Next, solution B was prepared by dissolving 1 g L-cysteine, 0.1 g ascorbic acid, 0.05 g potassium sorbate and 0.1 g sodium lauryl sulfate in 800 ml distilled water. Solutions A (200 ml) and B (800 ml) were then mixed and 3 *N* HCl was added to this mixture to adjust it to pH 3. This new disinfectant is a colorless and transparent liquid.

Table 1 Time taken to completely inactivate influenza A viruses following contact with the new disinfectant

Virus strain[a]	Time to complete disinfection (minutes)	
	1:9[b]	1:99[b]
H1N1 $10^{8.25}EID_{50}$/0.2 ml	>10	10
10-times diluted	10	10
H3N2 $10^{8.75}EID_{50}$/0.2 ml	10	10
10-times diluted	10	10
H4N6 $10^{8.50}EID_{50}$/0.2 ml	>10	10
10-times diluted	10	10
H5N3 $10^{8.25}EID_{50}$/0.2 ml	>10	2
10-times diluted	2	2
H6N2 $10^{7.75}EID_{50}$/0.2 ml	>10	10
10-times diluted	10	10
H7N7 $10^{8.25}EID_{50}$/0.2 ml	10	10
10-times diluted	10	10

EID_{50}, Median egg infectious doses (virus titre)

[a] H1N1, A/swine/Iowa/15/30; H3N2, A/Aichi/2/68; H4N6, A/duck/Czech/56; H5N3, A/whistling swan/Shimane/499/83; H6N2, A/duck/Massachusetts/3740/65; H7N7, A/whistling swan/Shimane/42/80

[b] Ratio of virus to the new disinfectant

This disinfectant was tested on six strains of influenza virus, namely, A/swine/Iowa/15/30 (H1N1), A/Aichi/2/68 (H3N2), A/duck/Czech/56 (H4N6), A/whistling swan/Shimane/499/83 (H5N3), A/turkey/Massachusetts/3740/65 (H6N2) and A/whistling swan/Shimane/42/80 (H7N7) [5, 6]. Prior to this investigation, these viruses were grown in the allantoic cavity of 10-day-old embryonated SPF hen's eggs for 2 days at 37°C. The allantoic fluid (virus fluid), which has a very high titre of hemagglutination (HA) activity, was collected and stored at −80°C. All virus strains were titrated by inoculating 10-day-old embryonated SPF hen's eggs via the allantoic cavity. Virus titres were expressed as median egg infectious doses (EID_{50}) [7]. The titres of all virus strains tested in these experiments were greater than $\log 10^{7.7}$ EID_{50}/0.2 ml (Table 1).

Two strengths of virus fluid were tested: undiluted and diluted tenfold in phosphate buffered saline (pH 7.2). 10- and 100-ml samples of both the diluted and undiluted virus fluids were poured into small tubes, made up to 1 ml with the new disinfectant solution, shaken carefully and left at room temperature. After incubations of 2, 10 and 60 min, respectively, the presence of surviving virus was determined by inoculating the virus fluid into the allantoic cavity of 10-day-old SPF hen's eggs.

Experiment 2

The acute toxicity of this disinfectant to cultured cell lines was investigated. CV-1 monkey kidney cells and Jurkat human lymphoma cells were seeded at densities of 5×10^5 cells/well and 1.0×10^5 cells/well, respectively, in 24-well tissue culture plates and incubated for 24 h in tissue culture media. Dulbecco's modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) was used to culture the CV-1 cells and RPMI-1640 supplemented with 10% heat-inactivated FBS was used for Jurkat. The new disinfectant and chrolhexidine gluconate as a positive control were added to duplicate wells at the final concentrations indicated in Table 2. The percentages of viable cells were determined by the trypan blue dye exclusion method 48 h later. Negative control wells were those without the test reagents. The percentages of growth inhibition were calculated by using the following formula:

Percentage growth inhibition = [(percentage dead cells in a test well) − (percentage dead cells in a control well)]/[100 − (percentage dead cells in a control well)].

Table 2 Cell toxicity of the different disinfectants tested

Test reagent	Final concentration (% of the working solution)[a]	Percentage inhibition of cell growth[b]	
		CV-1	Jurkat
New disinfectant	10.0	60.2 ± 5.9	48.4 ± 4.2
	1.0	28.4 ± 3.8	22.9 ± 10.5
	0.1	18.2 ± 3.3	4.6 ± 3.0
	0.01	13.1 ± 9.1	0
	0.001	5.3 ± 2.3	1.6 ± 1.5
	0.0001	0	0
Chrolhexidine gluconate (positive control)	100	100	100
	10	99.3 ± 0.9	100
	1.0	82.0 ± 1.2	87.0 ± 14.6
	0.1	63.2 ± 5.4	31.0 ± 18.6
	0.01	19.9 ± 7.5	15.6 ± 6.6
	0.001	0.6 ± 0.8	8.6 ± 7.6

[a] The new disinfectant is meant to be used undiluted, and thus the working concentration is 100%. At the lowest test dilution, the new disinfectant was added at 10% (v/v) strength to the culture media. The recommended working dilution for chrolhexidine gluconate for disinfection of the skin is 0.1–0.5%; therefore, for the highest test concentration, a Hibitane concentrate containing 5% (w/v) chrolhexidine gluconate was added at 1/50 (v/v) to the culture media

[b] Data are presented as the mean ± standard error of the mean (SEM), calculated from the results of three repeated experiments

Results and discussion

The results of experiment 1 (Table 1) show that all human, a swine and avian influenza A viruses belonging to the H1N1, H3N2, H4N6, H5N3, H6N6 and H7N7 subtypes lost at least 10^6 EID_{50} of their infectivity following contact with the new disinfectant for 10 min at room temperature,

thereby demonstrating that this disinfectant has a very strong anti-influenza virus activity. We did not use the H5N1 virus in this investigation for the following reason: it is difficult to get a sufficient high titre of H5N1 virus since the virulence of this highly pathogenic avian influenza virus is so severe that infected chick embryos died less than 16 h post inoculation and, therefore, the virus titre in allantoic fluid was generally low. We did succeed in generating a high pathogenicity with this H5N3 virus from an avirulent one by passaging it in chicks [5].

In experiment 2, as shown in Table 2, the cytotoxicity of the new disinfectant at the working concentration (100%) is weaker than that of chrolhexidine gluconate (0.1–0.5%).

Based on our results, the new disinfectant has a quick and strong anti-influenza viral activity, and its toxicity is rather weak. All human, swine and avian influenza viruses tested at a titre $>10^{7.7}$ completely lost their infectivity following contact with this new disinfectant for at least 10 min at room temperature. The acute toxicity of the new disinfectant is much weaker than that of chrolhexidine gluconate. We therefore suggest that this new disinfectant is both a safe and a promising disinfectant and that it can be used in any area where outbreaks of emergent infectious diseases, such as influenza, including that caused by the H1N1 subtype influenza virus, are occurring.

We are currently elucidating the underlying mechanisms of the anti-viral activity of this new disinfectant. We expect to find that the usual anti-bacterial activity of metallic ions is involved and also that the activity of this new disinfectant is stimulated by the existence of another unknown factor.

Our new disinfectant maintains the efficacy stated above for at least 3 years at room temperature (data not shown). It is reasonable to expect that this disinfectant will prove useful in preventing infection from the pandemic H1N1 subtype influenza virus and from other kinds of pathogens.

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