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Quantitative Evaluation of the Inactivation of Human Immunodeficiency Virus (HIV) by Antiseptics for the Oral Cavity

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Abstract Antiseptics used for the oral cavity were tested for the inactivation of human immunodeficiency virus (HIV). A quantitative bioassay system with a HTLV-I-carrying human T-cell line, MT-4 was used to evaluate virus inactivation. The tested compounds were as follows: povidone-iodine; benzethonium chloride; benzalconium chloride; chlorhexidine digluconate; methylrosaniline chloride; and ethacridine lactate. These compounds were tested at final concentrations of 1.0%, 0.1%, 0.01% and 0.001%, in the presence or absence of 10% fetal calf serum. The immunofluorescence assay for the HIV antigen showed that povidone-iodine, benzethonium chloride, and chlorhexidine digluconate were effective in the inactivation of HIV at concentrations for oral use. However, in the presence of protein, benzethonium chloride and chlorhexidine digluconate were ineffective at the indicated concentrations for oral use. Although benzalconium chloride was effective at 0.1%, this concentration cannot be used orally. Ethacridine lactate and methylrosaniline chloride were not effective at non-cytotoxic concentrations. The inhibitory effect of povidone-iodine was further tested by a HIV-specific plaque forming assay. The virus treated with concentrations higher than 0.1% of povidone-iodine formed no plaque. These results indicate that povidone-iodine is the most effective inactivator of HIV for use as an oral actiseptic in routine dental practice.

Key Words: Inactivation, HIV, Oral antiseptics, Povidone-iodine.

Introduction

Acquired immunodeficiency syndrome (AIDS) is considered to be one of the most serious diseases, chracterized by severe immune depression with deplition of the $T4^+$ subset (helper/inducer) of lymphocytes. Since human immunodeficiency virus (HIV) was identified as the etiologic agent of AIDS[1,2,3], many researchers have been looking for drugs that inhibit the infectivity and replication of HIV, e.g., 3'-azido-3'-deoxythymidine (AZT)[4,5] and 2',3'-dideoxynucleosides[6,7]. In spite of much effort, so far no therapeutic agent sufficiently effective to cure the clinical manifestation of AIDS has been established.

Dental personnel have a latent risk of HIV infection, because dental tereatment involves frequent bleeding and the personnel easily injure their hands and fingers with dental burs and pointed instruments. In fact, the rate of hepatitis B virus (HBV) infection of dental personnel is much higher than that of the general population[8]. Although HIV is not easily transmitted in routine dental

practices[9], the possibility of HIV transmission to the personnel still exsits. Infection control of HIV at the initial level is one of the most efficient ways for preventing AIDS prevalence. Several kinds of chemical disinfectants such as alcohols, hypochlorite, hydrogen peroxide, and glutaraldehydes were reported to be effective in the inacitivation of HIV[10,11]. However, these compounds produce adverse reactions when used on skin and mucosa, and cannot be used as oral antiseptics.

The accurate titration methods are essential for testing and evaluating the responses of HIV to chemical or physical agents. Therefore, various methods such as plaque forming assay, proliferation assay measuring cytopathic effect and indirect immunofluorescence (IF) assay for antigens of HIV were establishied[12,13]. This study presents a quantitative evaluation of the inactivation of HIV by several chemical disinfectans which have been used for the oral cavity.

Materials and Methods

Cells HTLV-I-carrying cell line, MT-4, which was highly permissive and susceptible to infection of HIV, was used in this study. In MT-4 cells, viral antigens were synthesized promptly upon HIV infection resulting in cellular death. The cells were cultured in RPMI 1640 containing 100 IU of penicillin per ml, 100μg of streptomycin per ml and 10% heat-inactivated fetal calf serum (FCS), at 37℃ in a CO_2 incubator and were subcultured twice a week.

Virus. HTLV-III B, one of the HIV strains, was obtained from the culture supernatant of its producer cells MOLT-4/$HIV_{HTLV\,III\,B}$, as described previously[14]. The titer of this virus preparation was determined by plaque forming assay (6×10^5 PFU/ml).

Oral antiseptics. The oral antiseptics tested in this study were as follows: 10% povidone-iodine, 10% benzethonium chloride, 10% benzalconium chloride, 5% chlorhexidine digluconate, 0.1% ethacridine lactate, and 2% methylrosaniline chloride. These disinfectants were diluted with phosphate-buffered saline (PBS) or PBS with 10% FCS to 1.0%, 0.1%, 0.01%, 0.001%, and 0% as a control, respectively.

Inactivation. The virus preparations (100 μl) were treated with 1 ml of each antiseptics for 1 min on ice, and then 10 μl of this virus preparation was inoculated into 1 ml of indicator cells (MT-4) suspension, which was adjusted to a concentration of 3×10^5 cell per ml. In this infectious condition, MT-4 cells were exposed to HIV to at a multiplicity of infection (MOI) of 0.0005 when the virus was treated with disinfectant-free PBS as a control. For the virus adsorption, cells were incubated for 1 hr at 37℃. The infected cells were wahsed once and resuspended in complete medium. The cells were cultured in a CO_2 incubator and a half of the medium was changed every 3 days.

Immunofluorescence (IF) method. Every 3 days until 21 days after infection, the HIV-infected MT-4 cells were smeared, dried and fixed with cold methanol for 3 min. The fixed cells were then incubated with 1:1,000 diluted human anti-HIV-positive serum (IF titer; 1:4,096) for 30 min at 37℃. The preparation was then washed for 15 min with PBS. The fluorescein-isothiocyanate-conjugated anti-human Ig-G (Dakopatts A/S, Copenhagen, Denmark) was then applied, and the preparation was incubated for 30 min at 37℃ and washed again with PBS. IF-positive cells were detected using a fluorescence microscope.

Plaque froming assay. To determine the residual infectivity titer of HIV after treating with povidone-iodine, a plaque forming assay was performed as described previously[12]. Briefly, to fasten MT-4 cells onto culture vessels, 35-mm polystyrene tissue culture dishes were coated with poly-L-lysine (PLL; mol. wt. 90,000, Sigma Chemical Co.). MT-4 cells (1.5×10^6 cells per ml) were then dropped onto each PLL-coated dish and were incubated for 1 hr at 37℃. Dishes were gently washed with PBS to remove unbound cells, and 100 μl of diluted virus preparation which was treated with various concentrations of povidone-iodine in PBS was slowly poured over the dishes after making a monolayer of MT-4 cells. The cells were incubated for 1 hr at 37℃ for virus adsorption. After incubation 1.5 ml of agarose overlay medium consistng of RPMI 1640 medium with 10% FCS, antibiotics, and 0.6% agarose (Sea Plaque agarose; FMC Co., Rockland, ME) was poured onto each dish. The dishes were incubated in a CO_2 incubator at 37℃ for 3 days, and 1.5 ml of agarose overlay medium containing neutral red was added, and incubated 3 more days for palque visualization. All experiments were carried out in triplicate.

Results

Infectivity of HIV was assessed for induction of virus-specific antigen which was detectable by IF in MT-4 cells after treatment with antiseptics (Fig. 1). The percentage of IF-positive cells was calculated every 3 days until 21 days after infection. When the virus was treated with effective concentrations of antiseptics, IF-positive cells did not appear during the 21 days incubation, whereas the proportion of IF-positive cells was about 5% at 3 days and nearly 100% at 6 days after infection with antiseptics-free virus preparation used as a control. Likewise, IF-positive cells appeared in the cultured cells infected with which virus was treated with ineffective doses of the various antiseptics (Fig. 1).

Povidone-iodine was effective at concentrations above 0.1% without any cytotoxicity to MT-4 cells and the effectiveness was not diminished even in the presence of protein (10% FCS). Although chlorhexidine digluconate and benzethonium chloride were effective at concentrations above 0.01%, cytotoxicity to MT-4 cells was observed at the 1.0% solution of these compounds. In the presence of protein, the effect of HIV disinfection was reduced for both compounds at the concentration of 0.01%. Benzalconium chloride was effective at concentrations above 0.1%, but cytotoxicity to MT-4 cells was also observed at the 1.0% solution. Ethacridine lactate did not inhibit HIV-specific antigen synthesis even in the 0.1% solution. Methylrosaniline chloride was also ineffective at the non-cytotoxic concentration to MT-4 cells

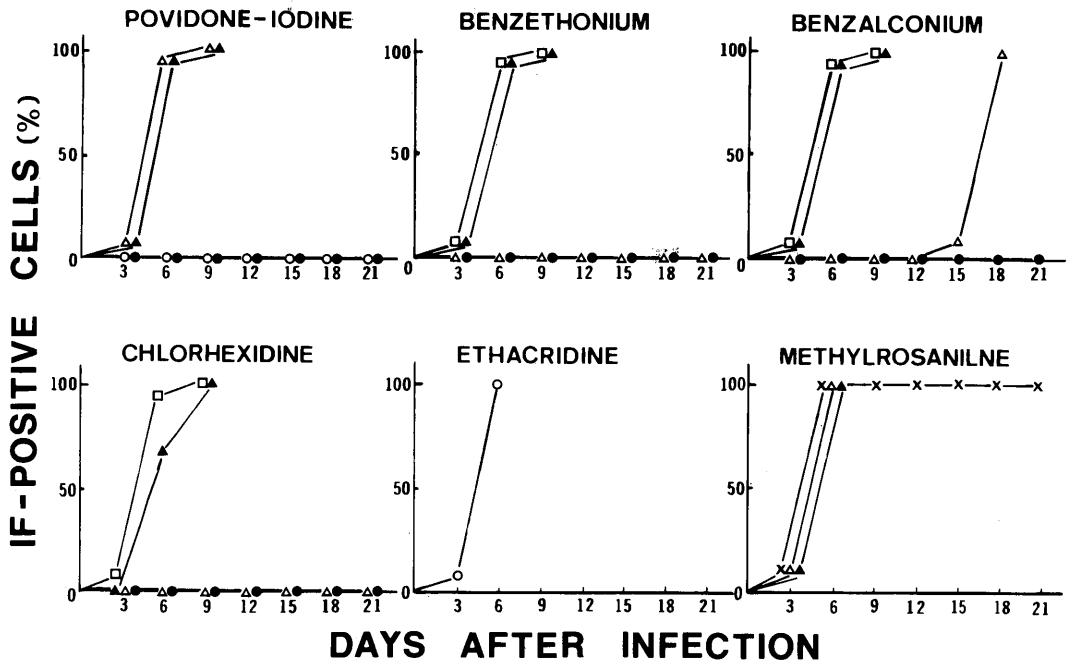

Fig. 1 Effect of various oral antiseptics on the inactivation of HIV as assessed by the induction of HIV-specific antigen in MT-4 cells detected by the immunofluorescence method. Antiseptics were diluted with PBS to concentrations of 0.1%(○), 0.01%(△) and 0.001%(□), respectively. To determine influence of protein, these disinfectants were also diluted with 10% FCS-containing PBS to 0.1%(●) and 0.01%(▲). Antiseptic-free virus preparation was used as a control (×). HIV-infected MT-4 cells were observed when the virus was treated with ineffective concentrations of the antiseptics and without the antiseptics. When HIV was treated with effective doses of the antiseptics, no antigen-positive cells were observed for 21 days after infection.

Table 1 INHIBITION OF THE HIV-SPECIFIC ANTIGENS SYNTHESIS IN MT-4 CELLS BY VARIOUS CONCENTRATIONS OF ANTISEPTICS

Antiseptics		Concentration of antiseptics (%)				
		1.0	0.1	0.01	0.001	0(cont.)
Povidone-	FCS(−)*	−§	−	+§§	+	+
iodine	FCS(+)**	−	−	+	+	+
Benzethonium	FCS(−)	CT#	−	−	+	+
chloride	FCS(+)	CT	−	+	+	+
Benzalconium	FCS(−)	CT	−	+	+	+
chloride	FCS(+)	CT	−	+	+	+
Chlorhexidine	FCS(−)	CT	−	−	+	+
digluconate	FCS(+)	CT	−	+	+	+
Ethacridine	FCS(−)	NT##	+	+	+	+
lactate	FCS(+)	NT	NT	+	+	+
Methylrosaniline	FCS(−)	CT	CT	+	+	+
chloride	FCS(+)	CT	CT	+	+	+

* Antiseptics were diluted with FCS-free PBS.
** Antiseptics were diluted with FCS-containing PBS.
§ No HIV-specific antigen was detected by IF during 21 days.
§§ HIV-specific antigen was detected by IF during 21 days.
# Cytotoxicity to MT-4 cells was observed.
Not tested.

(Table 1).

The effect of povidone-iodine was further examined by a plaque forming assay. HIV was treated with various concentrations of povidone-iodine in PBS for one minute on ice, before exposure to MT-4. Although 98 plaques were counted in the control virus preparation, treatment with povidone-iodine resulted in a decrease in the number of plaques in a dose-dependent fashion. Virus preparations treated with more than 0.1% povidone-iodine formed no plaque (Table 2).

Table 2 INACTIVATION OF HIV-INDUCED PLAQUE FORMATION BY POVIDONE-IODINE

Concentration(%)	Number of Plaques*
0.2	0
0.1	0
0.02	1.0±0.8
0.01	12.7±0.5
0.001	75.0±3.3
0**	98.0±4.6

*Mean ± S.D. per dish.
**HIV was treated with povidone-iodine free PBS.

Discussion

HIV is one of the retroviruses which contain a lipoprotein envelope[14] and is rather easily killed by various chemical and physical agents. For example, HIV is susceptible to inactivation by several physical conditions such as temperature[15,16], ultraviolet light[15,17] and gamma-ray irradiation[16]. Also, several disinfectants and sterilants such as alcohols, glutaraldehydes, hypochlorite, and hydrogen peroxide are effective in the inactivation of HIV[10,11] and have been used for decontaminating instruments and medical devices. However, these compounds pose problems in the case of mucous surfaces, especially oral mucosa because of their irritating nature. The disinfectants used as oral antiseptics have to be harmless to oral mucosa, safe when it is swallowed and effective by a short time treatment.

The six chemical disinfectants tested in this study are used as oral antiseptics during dental and oral surgical treatment. These compounds are classified into four groups. Benzethonium chloride and benzalconium chloride are popular quaternary ammonium compounds. The quaternary ammonium compounds cannot inactivate polioviruses[18], but can inactivate envelope-containing viruses such as herpes viruses[14].

Benzethonium chloride and benzalconium chloride can be used antiseptics for mucosa, including oral mucosa at the concentration between 0.01-0.02%. Chlorhexidine digluconate is also a popular antiseptic and classified as a surfactant. Chlorhexidine digluconate can be used as antiseptics for oral mucosa at concentrations between 0.02-0.05%. However, side effects such as anaphylaxis have been reported and this compound has subsequently been prohibited from mucosal use in Japan (Reported by The Japanese Ministry of Public Welfare in 1985.). Pindborg[19]described desquamation of oral epithelium by mouth rinsing with chlorhexidine digluconate; therefore its applicaition to the oral mucosa requires great care. Povidone-iodine is also a popular antiseptic and falls into the iodine group. It can inactivate poliovirus which is resistant to many disinfectants[18]. It can be used as an antiseptic for oral mucosa at the concentrations between 0.2-0.4%. Ethacridine lactate and methylrosaniline chloride are classfied as dyes. Ethacridine lactate is used for washing oral purulent lesion at the concentrations between 0.05-0.1%. Methylrosaniline chloride can be used as an oral antiseptic at the concentrations between 0.1-1.0%.

Among the tested compounds, povidone-iodine, benzethonium chloride, and chlorhexidine digluconate were effective in inactivation of HIV at the concentrations suitable for oral use with one minute treatment. Povidone-iodine appeared to be especially promising since it was effective even at 0.1% in inactivating HIV in the presence of 10% FCS. Asanaka and Kurimura[20] reported that povidone-iodine inhibited HIV infectivity at the concentration of 0.1% (100 ppm of available iodine) in 1% fetal bovine serum containing PBS with 15-second treatment. The data from this study closely matches their findings. Although 0.01% benzethonium chloride and 0.01% chlorhexidine digluconate also inactivated HIV, the effect of these disinfectants was conspicuously reduced in the presence of 10% FCS. It is well known that the effects of most disinfectants are reduced in the presence of protein such as human sera. Therefore, application of either benzethonium chloride or chlorhexidine digluconate appears to be doubtful effects in inactivation of HIV in the oral cavity.

HIV was detected in the saliva of AIDS related complex (ARC) patients and healthy carriers[21]. New outpatients do not always answer health-related questions truthfully in a dental office, and infective carriers are not always aware of their harboring disease. Silverman et al.[22] reported that many signs and symptoms of AIDS occur in the mouth in its early stages, such as oral candidiasis, hairly leukoplakia, Kaposi's sarcoma and lymphoma. Thus, undiagnosed AIDS patients may consult dentists because of oral problems. All dental personnel have the risk of exposure to HIV, although the probability appears to be minimal[9] at present. Therefore, it would be very beneficial to rinse patients' mouths with povidone-iodine (0.1%) before dental practice to minimize the chance of possible infection by HIV as well as by other viruses.

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