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Evaluation of an Antimicrobial Soap Formula for Virucidal Efficacy In Vitro against Human Immunodeficiency Virus in a Blood-Virus Mixture

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The virucidal efficacy of a health care personnel hand wash product containing 0.5% parachlorometaxylenol in a sodium C_{14-16} olefin sulfonate formula was evaluated in in vitro tests with human immunodeficiency virus type 1 (HIV-1) in the presence of 50% whole human blood. The HTLV-III_{RF} strain of HIV-1 was suspended in 50% medium–50% whole human blood and exposed to various dilutions of the hand wash product for 30 or 60 s. Following detoxification, residual infectivity was determined by a lytic cytopathogenic assay in MT2 cell cultures. No infectious HIV could be detected after a 30-s exposure to the hand wash product at dilutions of 1:5 and 1:10 and after a 60-s exposure at dilutions of 1:5, 1:10, 1:20, and 1:30. More than 99.9% of the virus was inactivated at these dilutions and exposure times.

The virus of acquired immunodeficiency syndrome is known to be a risk to health care workers (6, 8, 18). The causative agent, human immunodeficiency virus (HIV), is a member of the retrovirus class of enveloped viruses (2, 5, 9). Enveloped viruses are inactivated by a number of different surfactants and by a wide variety of antimicrobial compounds (1, 3, 14), and HIV has been shown to be inactivated by some of the same types of compounds (10, 11). On the basis of these observations, one would expect that antimicrobial soap formulations, such as health care personnel hand washes, would effectively inactivate HIV. However, inactivation of HIV by such formulations has not been demonstrated directly. A major reason for this lack of data is that hand wash formulations are cytolytic to cell culture assay systems and effectively preclude the assessment of viral cytopathic effects and the quantitation of viral inactivation.

In this paper, we present data on the in vitro inactivation of HIV in the presence of whole human blood by a health care personnel hand wash product. In addition, we describe the methods that were employed for separating cytotoxic antimicrobial components from infectious virus in virus-antimicrobial agent mixtures. These methods permitted the quantitation of virus inactivation.

MATERIALS AND METHODS

Virus. The RF strain of HTLV-III HIV (15, 19) was obtained from Robert C. Gallo, National Cancer Institute. Virus was produced by cultures of RF virus-infected H9 cells (12) and was concentrated from supernatant culture fluids by high-speed centrifugation (32,500 × *g* for 90 min) at 2°C. The virus pellet was suspended in 1/100 the original volume of complete RPMI 1640 medium containing 10% heat-inactivated (56°C, 30 min) fetal bovine serum and kept at 0°C.

Cell cultures and media. H9 cells were obtained from R. C. Gallo. Cultures were routinely grown in RPMI 1640 medium supplemented with 20% (vol/vol) fetal calf serum (heat inactivated at 56°C for 30 min), 100 U of penicillin per ml, 100 μg of streptomycin per ml, 2.5 μg of amphotericin B (Fungizone) per ml, and 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 7.2). MT2 cells (7) were obtained from Robert Shoemaker, National Cancer Institute, and were grown in the same medium but with 10% serum.

Hand wash product. The personnel hand wash product (Derma Cidol [Minnetonka Medical] contains 0.5% parachlorometaxylenol in a sodium C_{14-16} olefin sulfonate formula) was used at dilutions of 1:2.5, 1:5, 1:10, 1:15, 1:20, 1:30, 1:50, and 1:100 in Dulbecco phosphate-buffered saline (4).

Treatment of virus and sample. A portion (3.0 ml) of the virus suspension (diluted 1:10 in complete RPMI 1640 medium) was mixed with 3.0 ml of whole human blood known to be free of HIV antibody. Samples (0.25 ml) were dispensed into tubes, and 0.25 ml of the appropriate dilution of the hand wash product in phosphate-buffered saline was added and mixed (final dilutions of the hand wash product were 1:5, 1:10, 1:20, 1:30, 1:40, 1:60, 1:100, and 1:200). Sterile skim milk (0.5 ml of a 10% solution in water; Difco Laboratories) was added to each tube after 30 or 60 s in order to neutralize or retard virucidal and cytotoxic activities (16). Following the addition of skim milk, 0.6 ml of the virus-blood-hand wash product-skim milk mixture was immediately removed and added to a Sephadex column for separation of virus from the other components of the mixture.

Sephadex gel filtration. Sephadex gel filtration was performed by the general method of Blackwell and Chen (3). A 3-cm^3 column of Sephadex LH-60-120 was equilibrated with phosphate-buffered saline and precentrifuged at 600 × *g* for 3 min at 23°C to clear the void volume. Columns were loaded with approximately 0.6 ml of virus-blood-hand wash product-skim milk mixture and immediately centrifuged again under exactly the same conditions. The column filtrates were collected and diluted 10-fold serially for assay of infectivity.

Virus and cytotoxicity controls. Two 0.25-ml portions of the virus-blood mixture were placed in separate tubes, and 0.25 ml of phosphate-buffered saline was added to each.

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TABLE 1. Inactivation of HIV in 50% medium–50% whole human blood after a 30- or 60-s exposure to a personnel hand wash product

Final dilution of product	$-\log_{10}$ $TCID_{50}$[a]/0.05 ml			
	Cytopathic effects in MT2 cells (sample + virus) at[b]:		Cytotoxicity controls at:	
	30 s	60 s	30 s	60 s
1:5	≤2.5	≤2.5	2.5	2.5
1:10	≤2.5	≤2.5	2.5	2.5
1:20	3.0	≤2.5	2.5	2.5
1:30	3.5	≤1.5	1.5	2.5
1:40	3.83	2.34	1.5	2.5
1:60	3.83	2.5	1.5	1.5
1:100	4.67	2.34	1.5	1.5
1:200	5.67	4.67	≤1.5	1.5
Virus controls	>6.0	5.63[c]		

[a] $TCID_{50}$, 50% tissue culture infective dose.
[b] Toxicity precluded scoring of cytopathic effect at the next lower dilution.
[c] Average of 5.77 and 5.50.

After 30 or 60 s, 0.5 ml of skim milk was added to each tube, and the contents were filtered through the Sephadex column as described above. The cytotoxicity titer of each dilution of the product was determined by placing 0.25 ml of each dilution in a separate tube containing 0.25 ml of a 1:1 mixture of complete RPMI 1640 medium and whole human blood. Skim milk (0.5 ml) was added to each tube after 30 or 60 s, and the contents were gel filtered and assayed for cytotoxicity.

Infectivity assays. MT2 cells were indicator cells for infectivity assays (7, 17). MT2 cells were treated with Polybrene (2 μg/ml) for 30 min at 37°C, collected by centrifugation, and plated in 96-well culture plates at approximately 10^4 cells per well in 0.15 ml of medium. Dilutions of each of the test and control groups were inoculated (0.05 ml per well) into four replicate cultures of MT2 cells, and the cultures were scored for lytic cytopathic effects after 7 days of incubation at 37°C (17). Cytotoxicity was similarly assayed in quadruplicate. Viral and cytotoxicity titers were calculated by the method of Reed and Muench (13).

RESULTS

After a 30-s exposure of a blood-virus mixture to 1:5 and 1:10 dilutions of the personnel hand wash product, more than 99.9% of HIV infectivity was inactivated (Table 1). Under the experimental conditions that included the addition of skim milk at the 30-s point and Sephadex gel filtration, residual toxicity was still found at the lower dilutions. Despite this limitation, the reduction of infectivity was quantifiable.

The results of a 60-s exposure of blood-virus to the product are also shown in Table 1. More than 99.99% of virus was found to be inactivated by a 1:30 dilution of the product. In contrast to the results from the 30-s exposure, results from the 60-s test showed that dilutions of 1:20 and 1:30 were effective in inactivating virus. An increase in inactivation of virus with increasing exposure time was an expected result.

DISCUSSION

The results presented in this paper demonstrate the inactivation of HIV by a health care personnel hand wash formulation in the presence of whole human blood. Inactivation was quantifiable by titration of infectivity in a cytopathic effect assay after neutralization and separation of virus from product ingredients by gel filtration.

Inactivation of HIV by an antimicrobial soap was not surprising, because compounds that are cytolytic to mammalian cells would be expected to be virucidal for enveloped viruses above a threshold concentration (1, 10, 11, 14). It is exactly because of cytolytic effects, however, that virucidal effects are hard to measure. In addition, whole blood would be expected to protect virus by the presence of a large amount of membranous material. Nevertheless, by using a series of dilutions of the hand wash formulation, we were able to show concentration-dependent virucidal and cytotoxic effects under the test conditions. The 1:10 dilution of the personnel hand wash formulation is a typical concentration which represents normal-use exposure for products such as shampoos and liquid soaps.

The product under study was a sodium C_{14-16} olefin sulfonate formula containing 0.5% parachlorometaxylenol. The observed virucidal effects can be attributed to the known action of surfactants on enveloped viruses (1, 3, 10, 11, 14). No conclusions can be drawn regarding the virucidal activity of parachlorometaxylenol.

The experiments were designed to test exposure times of 30 and 60 s. However, residual virucidal activity after the addition of skim milk cannot be ruled out, and we do not have evidence in these experiments that virucidal activity was completely neutralized by skim milk. Nevertheless, it is still clear that very short exposure times were involved prior to gel filtration. Residual cytotoxicity was observed after gel filtration, but this does not necessarily mean that residual virucidal effects were also present, because different surfactants may vary in their effective concentrations. Tween 20 at a 1% concentration, for example, did not inactivate HIV (10). The use of Sephadex gel filtration to separate virus from product ingredients assumes that treatment with the product does not affect the ability of intact, infectious virus to pass through the gel. While we could not test for this directly, we have repeatedly observed noninfectious viral p24 in gel filtrates after treatment with a variety of disinfectants, indicating that disrupted viral components were able to pass through the gel (G. C. Lavelle and B. J. Bowden, unpublished observations). We believe it is unlikely that nondisrupted virus was selectively trapped in the gel. By using the general methods described here, it will be possible to study the virucidal efficacy and inactivation kinetics of different surfactants.

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