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Evaluation of Virucidal Compounds for Inactivation of Rhinovirus on Hands

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Antimicrobial liquids and foams were applied to the hands to determine their virucidal activity against rhinoviruses. Dilute solutions (1%) of iodine in ethyl alcohol or in water were most effective in causing inactivation of rhinovirus when applied immediately after viral contamination. Iodine also had a residual virucidal activity which persisted on the hands for up to 1 h. Less effective inactivation of rhinovirus was observed with foams containing 0.23% hexachlorophene and 58% ethyl alcohol and another containing 0.2% benzalkonium chloride and 50% ethyl alcohol. Ethyl alcohol alone or in a mixture with benzyl alcohol was the least effective preparation tested.

Persons with rhinovirus colds contaminate their hands and objects in their environment with nasal secretions containing the infecting virus (1, 6, 8). Under experimental conditions, susceptible volunteers acquire virus on the fingers from contact with contaminated hands and, in turn, infect themselves by self-inoculation of the nose or eye (5). If this sequence of events is important in the natural spread of rhinoviruses (4), it might be possible to develop means of interrupting viral spread by the hand contact/self-inoculation route. One approach would be to apply virucidal compounds to the hands of infected individuals and/or their contacts. Although mechanical removal of virus from the hands can be achieved by washing with soap and water, this is not always practical. Also, hand washing cannot be tested in double-blind experiments designed to determine if hand contact/self-inoculation is an important natural route of rhinovirus spread.

The purpose of the current investigation was to evaluate compounds which, when applied to the hands in small amounts, might inactivate virus on the skin surface. A method was developed to artificially contaminate hands and then measure viral inactivation after treatment with virucidal liquids and foams. Compounds which were found to be effective under these conditions will later be tested for their ability to interrupt spread of experimental and natural infection.

MATERIALS AND METHODS

Virus. The rhinoviruses used for hand contamination were laboratory strains of types 29 and 39 grown in human embryonic lung cell (WI-38) cultures and stored in multiple portions at −70°C. A 1:10 dilution of stock virus in Hanks balanced salt solution (HBSS) served as the hand contaminant in all experiments.

Hand preparations. The preparations selected for testing are shown in Table 1. A volume of 1.0 ml of a liquid or a 3-cm-diameter sphere of foam was applied to each hand in all experiments.

Viral contamination followed by application of virucidal preparation. Before each experiment, the hands of a volunteer were washed thoroughly with soap and water and then dried with paper towels. When the volunteer's hands were used for multiple experiments, washing was done between experiments. The fingertips of both hands were then contaminated with 0.3 ml of virus. Virus was spread over the fingers by gentle rubbing. After the fingertips had air-dried, compounds were applied, vigorously rubbed over the hand, and allowed to dry completely. The fingertips of each hand, up to approximately the second joint, were then separately rinsed into a sterile petri dish with a total of 2.5 ml of beef heart infusion broth with 1% bovine serum albumin per hand. A 1-ml amount of the rinse was recovered and added to 1.0 ml of collecting broth with antibiotics. An infectivity titration of a 0.1-ml sample of the rinse was performed in WI-38 cell culture tubes. As a control, the viral contaminated hands of the same volunteer were sampled after no treatment and/or after treatment with 1 ml of water per hand.

Application of virucidal preparation followed by viral contamination. Following the demonstration of an effective immediate virucidal action of iodine solutions on skin contaminated with rhinovirus, studies were done to evaluate the residual virucidal activity of these compounds. The test preparation was applied first, and after varying intervals of time, the hands were contaminated with virus and then sampled. The methods of sampling and culture were the same as those described above except that after the 0.1-ml sample of the hand rinse was obtained for an infectiv-

ity titration, the remainder of the 2.0 ml of the undiluted rinse was inoculated equally into four WI-38 cell culture tubes.

Procedure for removal of toxicity from specimens. The liquid hand preparations tested in the experiments exhibited no toxicity to the cell cultures when samples of hand rinses were directly inoculated into WI-38 cells. The foam preparations produced cytotoxicity and, therefore, a technique of high-speed centrifugation was used to recover virus from the hand rinse sample. A 1-ml amount of the hand rinse was diluted with 4.0 ml of HBSS and centrifuged at 150,000 × *g* for 3 h at 4°C. The supernatant was discarded, and the button was resuspended in 1.0 ml of collecting broth to reconstitute the original volume before centrifugation. Infectivity titrations were performed with this material. Control samples, using measured amounts of virus in collecting broth, showed no loss of infectivity with this procedure.

RESULTS

Viral contamination followed by application of virucidal preparations. The infectivity titer (50% tissue culture infective dose [$TCID_{50}$]) of the type 39 rhinovirus pool used to contaminate the hands varied between $10^{5.5}$ and $\geq 10^{6.5}$/ml. When fingertips of the contaminated hands were cultured after no treatment, the titers of recovered virus ranged from $10^{2.0}$ to $10^{4.5}$ $TCID_{50}$/ml (geometric mean titer [GMT], $10^{3.3}$) (Table 2). After treatment with 1 ml of water, viral titers ranged from $10^{1.5}$ to $10^{4.0}$ $TCID_{50}$/ml. The GMT after water treatment was $10^{2.5}$ $TCID_{50}$/ml.

A total of 19 tests were conducted with 70% ethyl alcohol. Virus was not eradicated in any of these experiments. The GMT was $10^{1.8}$ $TCID_{50}$/ml, a $10^{0.7}$ reduction from that measured with water treatment. In an additional 16 experiments, contaminated hands were rubbed with a paper towel soaked in 70% ethyl alcohol. Infectious virus was not eliminated from the fingertips by this treatment; however, the GMT of the residual virus was reduced to $10^{1.5}$ $TCID_{50}$/ml. Three other liquid preparations were tested for activity against rhinovirus type 39. Virus was undetected in 1 of 16 fingertip rinses when a mixture of 79% ethyl alcohol, 10% isopropyl alcohol, and 0.1% tetrabromo-*o*-cresol was employed, resulting in a GMT of $10^{1.3}$ $TCID_{50}$ of residual virus per ml. With a mixture of 3% benzyl alcohol and 62% ethyl alcohol, virus was not detected in 2 of 12 rinses, and the GMT of residual virus was $10^{1.3}$ $TCID_{50}$/ml. The placebo (10% ethyl alcohol) did not eradicate virus in any of 12 tests and had a residual GMT of $10^{2.2}$ $TCID_{50}$/ml, which was similar to that of water treatment.

Two foam preparations were tested. With a mixture of 0.23% hexachlorophene and 58% ethyl alcohol virus was not detectable in 21 of 42 tests. In 11 tests $>10^{1.0}$ $TCID_{50}$ of virus per ml was recovered. The GMT of residual virus was $10^{0.6}$ $TCID_{50}$/ml. Virus was not detected in 9 of 16 tests with a mixture of 0.2% benzalkonium chloride and 50% ethyl alcohol. Residual virus was recovered in a titer of $>10^{1.0}$ $TCID_{50}$/ml in four tests. The GMT of residual virus was $10^{0.8}$ $TCID_{50}$/ml.

Similar experiments were conducted with hands contaminated with rhinovirus type 29 (Table 3). With no treatment of the hands, type 29 rhinovirus was recovered in 8 of 10 tests. The GMT of the positive specimens was $10^{2.1}$ $TCID_{50}$/ml. In 24 of 28 tests with water treatment, the titer of residual virus was $>10^{1.0}$ $TCID_{50}$/ml. The GMT of the residual virus was $10^{2.1}$ $TCID_{50}$/ml.

Four active and two placebo liquids were tested. Virus could not be recovered from 3 of 14 fingertip rinses of hands treated with a mixture of 6% benzyl alcohol and 62% ethyl alcohol. The GMT of residual virus was $10^{1.4}$ $TCID_{50}$/ml. The placebo (10% ethyl alcohol) did not eradicate virus from any of 14 hands; the GMT of residual virus was $10^{2.3}$ $TCID_{50}$/ml. Virus was not detected in 1 of 10 rinses when an iodophor (poloxamer-iodine complex) was used; the GMT of residual virus was $10^{1.7}$ $TCID_{50}$/ml. A 1% aqueous iodine solution was very effective in eliminating infectious rhinovirus from fingertip rinses. Virus was undetectable in 16 of 18 tests, and only low concentrations of virus were measured in the two positive rinses. Residual virus was detectable in all 12 tests with the iodine placebo. The GMT of the residual virus was $10^{1.5}$ $TCID_{50}$/ml.

TABLE 1. *Active ingredients of preparations tested for virucidal activity against rhinoviruses*

Liquids
70% Ethyl alcohol
70% Ethyl alcohol (in paper towels)
79% Ethyl alcohol, 10% isopropyl alcohol
3% Benzyl alcohol, 62% ethyl alcohol
6% Benzyl alcohol, 62% ethyl alcohol
10% Ethyl alcohol (placebo)
Poloxamer-iodine complex containing 1% available iodine
1% Iodine, 2% potassium iodide
0.01% Iodine, 0.02% potassium iodide, (placebo)
Foams
0.23% Hexachlorophene by weight, 58% ethyl alcohol by volume
0.2% Benzalkonium chloride, 50% (wt/wt) ethyl alcohol
10% (wt/wt) ethyl alcohol (placebo)

TABLE 2. *Comparison of virucidal activity of selected preparations on hands contaminated with rhinovirus type 39*

Prepn	No. of tests	Viral titer[a] ($TCID_{50}$/ml) in rinse from fingertips									GMT[c]
		<1.0	1.0[b]	1.5	2.0	2.5	3.0	3.5	4.0	4.5	
Control											
No treatment	24	0			1		7	7	8	1	3.3
Water	40	0		2	8	10	13	6	1		2.5
Liquid											
70% Ethyl alcohol	19	0	3	7	3	4	1	1			1.8
70% Ethyl alcohol (in towels)	16	0	4	5	5	2					1.5
79% Ethyl alcohol, 10% isopropyl alcohol	16	1	5	4	4	2					1.3
3% Benzyl alcohol, 62% ethyl alcohol	12	2		7	2		1				1.3
10% Ethyl alcohol (placebo)	12	0		3	3	3	3				2.2
Foam											
0.23% Hexachlorophene by weight, 58% ethyl alcohol by volume	42	21	10	9	2						0.6
0.2% Benzalkonium chloride, 50% (wt/wt) ethyl alcohol	16	9	3		2	2					0.8

[a] Log base 10.
[b] Virus was detected in one of two tubes inoculated with this dilution.
[c] Log_{10} $TCID_{50}$/ml. GMT, Antilog of sum total of samples × their respective log titers/total of all samples.

TABLE 3. *Comparison of virucidal activity of preparations on hands contaminated with rhinovirus type 29*

Prepn	No. of tests	Viral titer[a] ($TCID_{50}$/ml) in rinse from fingertips							GMT[c]
		<1.0	1.0[b]	1.5	2.0	2.5	3.0	3.5	
Control									
No treatment	10	2		3		1		4	2.1
Water	28	0	4	7	5	6	4	2	2.1
Liquid									
6% Benzyl alcohol, 62% ethyl alcohol	14	3	3	2	1	3	1	1	1.4
10% Ethyl alcohol (placebo)	14	0		1	3	6	3	1	2.3
Poloxamer-iodine complex	10	1		4	3	1		1	1.7
Iodine (aqueous)	18	16	1	1					0.25
Iodine (placebo)	12	0	2	6	3		1		1.5
Foam									
0.2% Benzalkonium chloride, 50% ethyl alcohol	6	6							0.25
10% Ethyl alcohol (placebo)	6	0	1	3	1	1			1.5

[a] Log base 10.
[b] Virus was detected in one of two tubes inoculated with this dilution.
[c] Log_{10} $TCID_{50}$/ml.

No measurable virus was obtained from six fingertip rinses when a foam containing 0.2% benzalkonium chloride and 50% ethyl alcohol was employed. Residual type 29 virus was present (GMT $10^{1.5}$ $TCID_{50}$/ml) in six rinses from hands receiving placebo foam.

Residual virucidal activity of aqueous iodine solution. The iodine solution was tested for residual virucidal activity against rhinovirus type 29 at 0.25, 0.5, and 1 h (Table 4). In 14 of 16 tests with water treatment, the residual viral titer was $>10^{1.0}$ $TCID_{50}$/ml, and the GMT varied from 1.4 to 2.1. In contrast, with iodine treatment, a residual titer of $>10^{1.0}$ $TCID_{50}$/ml was

TABLE 4. *Residual virucidal activity of aqueous iodine solution on hands contaminated with rhinovirus type 29*

Time interval (h)	No. of tests	Viral titers[a] ($TCID_{50}$/ml) in rinse from fingertips							GMT[c]
		<0.5[b]	0.5[b]	1.0	1.5	2.0	2.5	3.0	
Water									
0.25	6				5	1			1.4
0.5	6			1	3	2			1.4
1.0	4			1		1	1	1	2.1
Iodine									
0.25	18	12	3	3					0.2
0.5	18	12	4	2					0.2
1.0	8	2	1	4	1				0.6

[a] Log base 10.

[b] At these dilutions, four tubes each were inoculated with 0.5 ml of undiluted rinse.

[c] Log_{10} $TCID_{50}$/ml.

measured in only 1 of 44 tests and the GMT ranged from 0.2 to 0.6 for the three time intervals.

DISCUSSION

Klein and Deforest have established a classification in which viruses are placed into three different categories based on their susceptibility to chemical germicides (7). One group, the lipophilic viruses, have an outer envelope containing lipid, whereas a second group, although they do not contain a lipid envelope, can be inactivated by lipophilic virucides in the same manner as the lipophilic group. The picornavirus family, of which rhinoviruses are members, fall in the category of hydrophilic or nonenveloped viruses, possessing an inner core of nucleic acid and an outer protein coat. This third group of viruses has a strong resistance to lipophilic compounds and is, thus, resistant to many commonly used germicides.

In this investigation, selected virucidal compounds were evaluated by a method developed for testing preparations which were applied to the hands. The ideal compound for this use would be safe and nonirritative to skin after multiple applications and would have both immediate and prolonged residual virucidal activity, thus circumventing the need for mechanical removal of virus by frequent washing.

One percent iodine was the most effective of the compounds tested, showing both immediate and residual activity against type 29 rhinovirus. Virucidal activity of iodine has been recognized (2, 3), and its effectiveness against rhinovirus, when applied to the skin, is not surprising. The practicality of using hand preparations containing iodine is questionable, however, because of skin staining and the occasional occurrence of hypersensitivity (2, 3, 9). The iodine solutions can be used to conduct double-blind experiments in families to evaluate the importance of hand contact spread of rhinovirus. A placebo prepared from 2% food coloring and 0.01% iodine plus 0.02% potassium iodide had no virucidal activity and was indistinguishable from the active solution in color, skin staining, and odor. The staining property of iodine solutions, while a disadvantage for their ultimate practical use, does provide a way of assessing compliance when tested under field conditions.

Of the other preparations tested, only combinations of hexachlorophene and ethyl alcohol in foam and benzalkonium chloride and ethyl alcohol in foam showed substantial inactivation of rhinovirus on the hands. While ethyl alcohol alone is known to have some activity against hydrophilic or nonenveloped viruses (7, 11), its effect against rhinovirus, as shown in the current study, is limited. Hexachlorophene is insoluble in water (10), and its effect against hydrophilic viruses when used alone is unknown. Benzalkonium chloride is a markedly lipophilic compound which is ineffective against hydrophilic viruses (7). The reason for the relatively good rhinovirucidal action of foams containing either of these two compounds combined with ethyl alcohol is unknown. There is the possibility that a synergistic virucidal effect was produced by the combination of the compounds. Although a remote possibility, the mechanical effect of the breaking foam may have enhanced the virucidal activity of the ethyl alcohol.

Up to the present time, little work has been done to evaluate virucidal activity of compounds on the hands. If future epidemiological studies indicate that hand contact/self-inoculation is an important way in which rhinovirus colds spread, virucidal hand preparations should receive further investigation as a way of controlling these infections. The amount of rhinovirus involved in transmission of infection may not be large (5), and application of compounds with even moderate virucidal activity to the hands may be sufficient to interrupt the chain of transmission at this point. Safe and effective hand preparations can probably be developed for this use.

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