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Rhinovirus inactivation by nasal tissues treated with virucide

Gregory F. Hayden[1,*], Jack M. Gwaltney, Jr.[2], Deborah F. Thacker[2] and J. Owen Hendley[1]

[1]*Department of Pediatrics, and* [2]*Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, VA 22908, U.S.A.*

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Summary

Rhinovirus colds may be transmitted by hand-to-hand contact followed by self-inoculation of nasal and/or conjunctival mucosa with virus contaminating the fingertips. The purpose of this study was to determine whether impregnation of nasal tissues with virucidal compounds could prevent rhinovirus from passing through the tissue and thus provide a means of preventing hand contamination during nose blowing. Paper tissues treated with a combination of citric acid, malic acid, and sodium lauryl sulfate were compared to placebo tissues containing sodium saccharin. Recovery of infectious virus was significantly reduced by passage of the virus-containing medium through virucidal versus placebo tissue (1/18 vs. 17/18 respectively, $P < 0.001$, Fisher exact test). The virucidal effect of treated tissues was demonstrated for multiple rhinovirus serotypes suspended in either cell culture medium or nasal mucus. Virus contained in mucus from infected volunteers was also inactivated.

common cold; rhinovirus; virucide; acute respiratory disease; nasal tissues

Introduction

Rhinovirus colds may be transmitted by hand-to-hand contact followed by self-inoculation of nasal and/or conjunctival mucosa with virus contaminating the fingertips [1–3]. Previous attempts to block this route of transmission have stressed inactivation

* To whom all correspondence should be addressed: Department of Pediatrics - Box 501, University of Virginia School of Medicine, Charlottesville, VA 22908, U.S.A.

of virus after it has been deposited on the hands [4–7], but effective virucidal preparations for hand treatment are not yet available. Aqueous iodine has been shown effective in inactivating rhinovirus on skin and in blocking hand-transmission of experimental rhinovirus infection [4,6], but its staining properties make it unsuitable for practical use. Virucidal hand lotion containing 2% glutaric acid is safe and cosmetically acceptable, but is not effective against all rhinovirus serotypes [5,7].

The sequence of viral transmission by hand-contamination/self-inoculation could potentially be interrupted at the first step, the deposition of virus on the hands of the infected individual. The excess of nasal secretions during a common cold generally prompts frequent nose blowing with resultant finger contact with nasal mucus, so that virus has been commonly found on the hands of adults with rhinovirus colds [3,8,9]. Virucidal nasal tissues might reduce the spread of colds by blocking hand contamination during nose blowing. The purpose of this study was to determine whether the passage of infectious rhinovirus through paper tissues could be prevented by impregnation of the tissues with virucidal compounds.

Materials and Methods

Tissues

Five types of paper tissue and a cloth handkerchief were tested. One paper tissue was commercial (Kleenex®) two-ply tissue purchased in a local supermarket. Four different types of experimental paper tissue were supplied by the manufacturer (Kimberly Clark Corporation, Neenah, WI) in coded boxes as follows: two-ply untreated, three-ply untreated, two-ply treated with virucide, and three-ply treated with virucide. Because of the known inactivation of rhinovirus in an acid environment, the manufacturer evaluated the virucidal properties of several organic acids, alone and in combination. On the basis of these experiments, the tissues were treated with an aqueous solution containing citric acid, malic acid, and sodium lauryl sulfate. Each square inch of tissue contained approximately 3.5 mg of citric acid, 1.7 mg of malic acid and 0.7 mg of sodium lauryl sulfate. Unpublished observations have shown that rhinovirus type 14 is dissociated by exposure to this mixture, and that the virus is killed in the tissue within seconds of exposure (R. Rueckert, personal communication, 1984). The untreated tissues were prepared in a similar manner with a solution of sodium saccharin (2.5 mg per square inch). The investigators were blinded to the nature of the tissues until completion of the experiments. In one experiment, a cotton cloth handkerchief was used.

In a separate experiment, the tissues were washed before use with phosphate buffered saline (PBS, pH 7.2) to determine whether the virucidal compounds could be eluted from the treated tissues. In this experiment each tissue was draped over a funnel, and 4.0 ml of PBS was dripped onto the superior surface. Each tissue was allowed to dry overnight before testing.

Viruses

Eight strains of different rhinovirus serotypes suspended in cell culture medium

were used. Most of the experiments utilized laboratory stock strains of rhinovirus serotypes which had been stored frozen at -70°C in medium containing 2% fetal bovine sera. These eight strains included seven numbered serotypes (T 2, 4, 16, 33, 39, 44, 75) and also rhinovirus strain HH, which is an acid-sensitive, chloroform- and iodoxuridine-resistant strain not neutralized by antisera to the 89 numbered rhinovirus types. The viruses were thawed and then used either undiluted or mixed artificially with an equal volume of nasal mucus from a single healthy adult. Mucus from this individual had been pretested to determine that it did not contain neutralizing antibody to the rhinovirus serotypes with which it was mixed. In other experiments, nasal mucus from four volunteers infected with one of two rhinovirus serotypes (T 39 or HH) was collected by blowing the nose into beakers which were kept on wet ice before use.

Virus exposure to nasal tissue

The tissue was draped over an opened 100 × 15 mm plastic petri dish so that the center of the tissue was in contact with the bottom of the dish. The virus suspension was then pipetted onto the superior side of the tissue. The volume of the suspension applied in the standard test was 0.1 ml, but larger volumes of up to 2.0 ml were used in other experiments. Within 3–5 s of application of virus, the tissue was lifted out of the dish and discarded. Visible wetting of the plastic surface contacting the inferior side of the tissue occurred during this brief interval. The dish was then rinsed with 1.0 ml of virus collecting broth (VCB) composed of beef heart infusion broth containing 1% bovine serum albumin and antibiotics. The recovered fluid was tested for the presence of infectious virus by inoculation of 0.2 ml of undiluted fluid and 0.1 ml of a 1 : 10 dilution in Hanks' balanced salt solution (HBSS) into two screw-capped tube cultures of human embryonic fibroblasts, MRC-5 strain (M.A. Bioproducts; Walkersville, MD). Tubes were read daily and judged positive if typical rhinovirus cytopathic effect developed. Cultures were examined for 2 weeks before being discarded as negative. The amount of viral growth was expressed in tissue culture infectious doses $(TCID)_{50}$ per 0.1 ml of inoculum as follows: 0 = no virus detected in undiluted collecting broth, 10^0 = virus detected in one of the two tubes inoculated with undiluted collecting broth, $10^{0.5}$ = virus detected in both tubes inoculated with undiluted collecting broth, 10^1 = virus detected in one of the two tubes inoculated with diluted collecting broth, and $\geqslant 10^{1.5}$ = virus detected in both tubes inoculated with diluted collecting broth.

Results

Virus suspended in cell culture medium and nasal mucus

Infectious rhinovirus suspended in cell culture medium consistently passed through the untreated commercial tissue and the two- and three-ply tissues containing the placebo substance (Table 1A). Twenty-six of 27 specimens were positive. Viral passage through the three-ply placebo tissue appeared slightly diminished compared to that with the two-ply placebo tissue ($P = 0.06$, one-sided sign test). In contrast,

TABLE 1

Rhinovirus infectivity after passage through virucidal nasal tissues

Rhinovirus		Viral titer ($TCID_{50}$/0.1 ml) after passage through designated tissue				
Type	Titer ($TCID_{50}$/0.1 ml)	Commercial 2-ply tissue	Experimental tissues			
			Untreated		Virucidal	
			2-ply	3-ply	2-ply	3-ply
A. Virus diluted in cell culture medium						
2	$10^{5.0}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	0	0
2	$10^{4.0}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	0	0
4	$10^{3.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$10^{1.0}$	0	0
16	$10^{3.0}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$10^{0.5}$	0	0
33	$10^{2.5}$	$10^{0.5}$	$10^{0.5}$	$10^{0.5}$	0	0
39	$10^{4.0}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	10^{0}	0
44	$10^{3.0}$	10^{0}	$10^{0.5}$	0	0	0
75	$10^{3.0}$	$10^{0.5}$	$10^{0.5}$	10^{0}	0	0
HH	$10^{4.0}$	$\geqslant 10^{1.5}$	$10^{1.0}$	$10^{0.5}$	0	0
B. Virus diluted in nasal mucus						
2	$10^{5.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	0	0
2	$10^{5.0}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	0	0
16	$10^{2.5}$	$10^{0.5}$	$10^{0.5}$	$10^{0.5}$	0	0
16	$10^{2.5}$	$10^{1.0}$	$\geqslant 10^{1.5}$	$10^{0.5}$	0	0
39	$10^{4.0}$	$\geqslant 10^{1.5}$	$10^{1.0}$	$\geqslant 10^{1.5}$	0	0
39	$10^{3.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	0	0

infectious virus was recovered after exposure to the tissues impregnated with virucide in only one of 18 tests ($P = < 0.001$, Fisher exact test). In one trial with the two-ply treated tissue, type 39 rhinovirus was detected in one of two tubes. Thus, the effect of impregnation of the tissue with virucide appeared much greater than the effect of adding an additional ply to the tissue. In the experiment using the cotton handkerchief, type 39 rhinovirus was detected in both tubes inoculated with the diluted suspension in the two experiments performed.

Rhinovirus which was artificially mixed with nasal mucus was recovered in all 18 specimens collected after passage through the commercial or placebo-treated tissue (Table 1B). No virus was detected in 12 specimens obtained using treated tissues.

Effect of inoculum volume and washing

To determine if the virucidal effect of the treated tissues could be overwhelmed with larger volumes of viral inoculum, the experiment was repeated using virus in cell culture medium with inoculum volumes of 0.5, 1.0 and 2.0 ml (Table 2). With type 2 rhinovirus, no virus was recovered even after passage of 2 ml of inoculum through the tissues. The infectivity of type 39 rhinovirus was also substantially reduced although virus was recovered in three of 14 specimens.

TABLE 2

Effect of inoculum volume on killing of rhinovirus by virucidal nasal tissues

Rhinovirus			Viral titer ($TCID_{50}$/0.1 ml) after passage through designated tissue				
Type	Titer ($TCID_{50}$/0.1 ml)	Inoculum volume (ml)	Commercial 2-ply tissue	Experimental tissues			
				Untreated		Virucidal	
				2-ply	3-ply	2-ply	3-ply
2	$10^{4.5}$	0.5	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	0	0
	$10^{3.0}$		$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	0	0
	$10^{4.5}$	1.0	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	0	0
	$10^{5.0}$		$\geqslant 10^{1.5}$	ND	ND	0	0
	$\geqslant 10^{5.5}$	2.0	$\geqslant 10^{1.5}$	ND	ND	0	0
39	$10^{4.0}$	0.5	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	10^{0}	0
	$10^{4.5}$		$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	0	0
	$10^{4.5}$		$10^{1.0}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	10^{0}	0
	ND			$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	0	0
	$10^{4.0}$	1.0	$\geqslant 10^{1.5}$	ND	ND	0	0
	$10^{4.5}$		$\geqslant 10^{1.5}$	ND	ND	0	0
	$10^{5.0}$	2.0	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	0	$\geqslant 10^{1.5}$

ND = not done or test contaminated.

In an experiment in which treated tissues were prewashed with 4 ml of PBS before the viral inoculum was applied, virus was recovered in all four of the samples collected.

Mucus from infected volunteers

Nasal mucus from four volunteers infected with rhinovirus strain HH or type 39 was applied to two-ply tissue (Table 3). Samples were collected from both the superior (nasal) side of the tissue and the fluid passing through the tissue to the bottom of the petri dish. With strain HH, no virus was detected in fluid which passed through either virucidal or placebo tissue. Rhinovirus type 39 was recovered from the petri dish in all three experiments with the placebo tissue but from none of the three with the virucidal tissue.

Strain HH virus was detected in the mucus remaining on the superior side of the tissue in three of five samples from both the virucidal and placebo tissues. Rhinovirus type 39 was detected on the superior surface of all three placebo tissue specimens and two of three virucidal tissue specimens.

Discussion

In view of the substantial morbidity caused by common colds, a simple and effective

TABLE 3

Effect of virucidal nasal tissue on rhinovirus recovery from nasal mucus from infected volunteers

Rhinovirus type	Quantity of mucus (ml)	Virus titer ($TCID_{50}$/0.1 ml) in indicated specimens			
		Mucus on superior side of tissue		Fluid traversing tissue	
		Untreated tissues	Virucidal tissues	Untreated tissues	Virucidal tissues
HH	0.1	$10^{1.0}$	$10^{1.0}$	0	0
	0.3	$10^{0.5}$	$10^{0.5}$	0	0
	0.3	0	0	0	0
	0.5	$10^{0.5}$	$10^{0.5}$	0	0
	0.5	0	0	0	0
39	0.1	$\geqslant 10^{1.5}$	$\geqslant 10^{1.5}$	$10^{0.5}$	0
	0.2	$\geqslant 10^{1.5}$	0	$10^{0.5}$	0
	0.3	$\geqslant 10^{1.5}$	$10^{1.0}$	$10^{0.5}$	0

method of reducing the spread of illness would be of great public health importance. Earlier studies have suggested that hand-to-hand transmission may be an important method of spread [1–3]. In this study, it was shown that infectious rhinovirus readily passed through commercially available nasal tissues and a cotton handkerchief, and that treatment of tissues with virucidal compounds prevented passage of virus through the tissue.

The reduction of viral recovery when virucide was present was much more pronounced than that obtained by adding an additional ply of tissue. Further, the effectiveness of the virucidal treatment was observed whether the tissue received stock virus suspended in cell culture medium or nasal mucus, or actual virus-containing nasal mucus from infected volunteers. Relatively large volumes of viral inoculum were required to overwhelm the effectiveness of the treated tissues (Table 2); however, the virucidal activity could be eliminated by washing the tissue. The latter would not be expected to be a problem during use under natural conditions. Virus in mucus remaining on the nasal side of the treated tissue was not inactivated after a 3–5 s exposure to the tissue.

The use of nasal tissues during a cold is a widely accepted form of personal hygiene. Further studies are needed to determine whether tissues treated with virucidal compounds will be effective in preventing viral contamination of fingers during nose blowing and whether their use will interrupt transfer of rhinovirus by the hand contamination/self-inoculation route. If so, virucidal nasal tissues may prove useful as a practical and acceptable way to reduce transmission of rhinovirus colds.

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