Lack of Effect of an Interferon Inducer, \(N,N\)-Dihexadecyl-\(m\)-Xylylenediamine, on Rhinovirus Challenge in Humans

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Received for publication 30 November 1978

CP-28,888-27 and placebo nasal sprays were compared in 62 normal volunteers challenged with rhinovirus type 13 or 21 in two randomized, double-blind studies. Half of the subjects received CP-28,888-27 and half received nasal placebo administered at 24, 20, and 16 h before challenge and 4 and 8 h after challenge. In each study, the number of subjects shedding virus in nasal washes, the number developing fourfold or greater serum antibody responses, and the number developing afebrile or febrile upper respiratory tract illness were not significantly different comparing subjects given CP-28,888-27 and those given placebo. Interferon was detected in nasal washes from 5 of 15 volunteers tested in the CP-28,888-27 group compared to 2 of 15 volunteers from the placebo group.

The use of exogenous interferon or interferon inducers to control respiratory viral infections appears attractive because of the diversity of viral families and serotypes that produce common cold syndromes. Some success has already been achieved by this approach. In studies in volunteers, the intranasal application of large quantities of human leukocyte interferon has been shown to reduce the symptoms of rhinovirus, but not those of influenza B illness, and topical application of polynosinic acid:polycytidylic acid was shown to have some effect in reducing incidence of rhinovirus infections (5, 8).

Over the past few years a new class of low-molecular-weight interferon inducers has been developed. The first to be tested was an alkyl-substituted propanediamine referred to as CP-20,961 (1–3, 6, 9, 10, 12). More recently, a related compound, \(N,N\)-dihexadecyl-\(m\)-xylylenediamine, purported to be more effective in animals than CP-20,961 and referred to as CP-28,888-27, has been developed (John Niblack, personal communication). We evaluated this drug in experimental rhinovirus infection in volunteers.

MATERIALS AND METHODS

Two separate studies were performed at different sites: (i) 32 volunteers were studied in Gainesville, Fla.; and (ii) 30 volunteers were studied in Rochester, N.Y. Volunteers. Subjects were healthy adult males between the ages of 19 and 30 years. Procedures used to recruit the volunteers insured their freedom of choice to participate, and volunteers gave written informed consent after they received an explanation of the risks involved. Volunteers were isolated for 8 days, beginning the day before virus challenge. Before and at the end of the study, volunteers underwent complete history, physical examination, complete blood count, and SMA-12. Volunteers were randomly allocated to the placebo or drug group. Analysis of age, race, and body weight revealed no significant difference between the two groups.

Drugs. The substituted xylylenediamine, CP-28,888-27, was supplied by Central Research, Pfizer, Inc., in multiple-dose spray containers with 50 mg of drug per ml in vehicle. Because of its insolubility, the drug was fused with equal weights of polysorbate 80 and glycerol and then homogenized in hot buffered (pH 7) 0.14 M NaCl, yielding a particle size <2 \(\mu\)m in diameter in the homogenate. The spray apparatus was calibrated to deliver 1 ml/dose in large particles (>25 \(\mu\)m in diameter) to the nasal mucosa. Identical containers with only the vehicle were provided. Drug or placebo was administered at 24, 20, and 16 h before virus challenge and at 4 and 8 h after challenge.

Challenge virus. Rhinovirus inocula were prepared in WI-38 human lung fibroblasts and were shown by inoculation of various tissue cultures after specific neutralization to be free of extraneous viruses (7). Rhinovirus type 21 was used in the study performed in Gainesville, and type 13 was used in Rochester. The challenge virus was given to the volunteers by nose drops, 0.25 ml in each nostril, containing three 50% tissue culture infectious doses (TCID\(_{50}\)) of rhinovirus type 21 or 80 TCID\(_{50}\) of type 13.

Specimens obtained. Nasal washings, using 6 to 10 ml of tissue culture medium or saline infusion broth, were obtained each morning for virus isolation. Blood was obtained before the start of the study and 3 to 4 weeks after challenge for antibody determinations.

Clinical evaluation. Oral temperatures were recorded at 8 a.m., 12 noon, 4 p.m., and 8 p.m. Volunteers were examined daily by a physician who did not know their treatment group status. They were questioned and examined for signs and symptoms referable to the
respiratory tract, and severity of each sign or symptom was recorded on a basis of 1+ to 4+ (e.g., nasal obstruction, cough, etc.). When total symptom scores were analyzed, prechallenge scores on days 0 and -1 were subtracted from the postchallenge scores for each symptom to eliminate minor "background" complaints. In addition, physicians made overall evaluations for presence of illness and site of involvement (e.g., rhinitis, pharyngitis, systemic illness, tracheobronchitis) (1).

Virus isolation. A 0.2-ml amount of the nasal washing obtained each day was inoculated onto WI-38 cell cultures, as previously described (1). The identity of the first isolate from each volunteer was confirmed by neutralization tests, using specific hyperimmune guinea pig serum.

Antibody determination. Neutralizing antibody was measured in sera, using WI-38 cell cultures and 10 TCID₅₀ of homotypic rhinovirus (1).

Interferon assay. Nasal secretions were collected each morning by instilling 10 ml of lactated Ringer solution into the nasopharynx and collecting the effluent fluid plus discharge by blowing. This material was homogenized in glass tissue grinders, clarified by centrifugation, and concentrated 10-fold by air drying in dialysis tubing. Specimens thus concentrated were treated with ultraviolet irradiation. Testing for interferon was performed in human neonatal foreskin fibroblast cell cultures challenged with vesicular stomatitis virus as described by Petralli et al. (11). Each assay incorporated a standard interferon preparation that contained 600 IU as compared with the human interferon standard A (10,000 U/ml) obtained from the Medical Research Council, England. Titers are expressed as recioicals of dilutions inhibiting 50% plaque-forming units. Previous studies have shown that the vesicular stomatitis virus inhibitor detected in nasal secretions of volunteers undergoing rhinovirus challenge is heat and acid resistant but sensitive to trypsin (1).

RESULTS

Virological responses. Virus isolation, serum antibody, and nasal interferon responses to rhinovirus challenge are shown in Table 1. As indicated, similar proportions of volunteers in each group became infected as determined by virus isolation and/or antibody response. There was a slightly higher infection rate among volunteers in the placebo group inoculated with 80 TCID₅₀ of rhinovirus type 13, 93%, as compared to those given placebo and 3 TCID₅₀ of rhinovirus type 15, 81%. Within each study (virus inoculum), responses were similar except for a lower rate of isolation of virus among volunteers treated with placebo and challenged with type 21 (P > 0.05, chi square). Analysis of numbers of volunteers shedding virus by the day after inoculation also revealed no significant differences in each study (not shown).

Nasal secretion interferon. Interferon levels were determined only on volunteers challenged with rhinovirus type 13. Five of 15 in the CP-28,888-27 group and 2 of 15 in the placebo group had interferon detected in their nasal washes. The distribution of interferon-positive nasal wash specimens according to day after inoculation is shown in Table 1. The most frequent day of detection of interferon was day 5 after inoculation. Titers were low and ranged between 5 and 10 IU on each specimen. Most patients had single specimens containing interferon except for two patients in the CP-28,888 group, each of whom had two positive specimens.

Clinical responses (Table 2). Illness occurred only in infected volunteers. There were no significant differences in either study between placebo and CP-28,888-27 groups for numbers of volunteers developing illness or febrile illness (temperature ≥37.7°C), although the rate of illness in the placebo group in the study using rhinovirus type 21 was lower than for the other groups. In spite of the lower rate of illness, severity of illness was somewhat greater in this group, as indicated by the number of volunteers with fever and the higher peak symptom score. No significant differences were detected in either study between the placebo and CP-28,888-27 groups in regard to average daily symptom scores among ill volunteers (Student's two-sample t test). Comparison of the average severity score for

<table>
<thead>
<tr>
<th>Rhinovirus</th>
<th>Dose (TCID₅₀)</th>
<th>Group</th>
<th>No. of volunteers</th>
<th>Virus isolation</th>
<th>≥4-Fold serum antibody response</th>
<th>Either criteria</th>
<th>Interferon response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 13</td>
<td>80</td>
<td>CP-28,888-27</td>
<td>15</td>
<td>13</td>
<td>14</td>
<td>14</td>
<td>No with interferon in NW*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Placebo</td>
<td>15</td>
<td>13</td>
<td>14</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Type 21</td>
<td>3</td>
<td>CP-28,888-27</td>
<td>16</td>
<td>9</td>
<td>13</td>
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<td>Placebo</td>
<td>16</td>
<td>4</td>
<td>12</td>
<td>13</td>
<td>NT</td>
</tr>
</tbody>
</table>

* NW, Nasal wash.
* NT, Not tested.
each symptom and the average duration of each symptom revealed no differences between placebo and CP-28,888-27 groups for either study.

**DISCUSSION**

In the present studies, CP-28,888-27, a low-molecular-weight interferon inducer, was shown to exert no significant effects on virus shedding, illness responses, and serum antibody responses in experimental rhinovirus infection in volunteers. The result is strengthened by the fact that studies performed in two different sites using different virus inocula gave similar results. Rhinovirus type 13 is known to be sensitive to interferon (4), and the same inoculum had been used in studies with CP-20,961 which demonstrated an effect on severity of illness (1).

The result occurred despite the fact that a related compound, CP-20,961, had stimulated interferon in nasal washes and exerted prophylactic effects in volunteers against rhinovirus challenges in several different studies (1–3, 9, 10, 12). In addition, CP-28,888-27 appeared to be a more potent interferon inducer than CP-20,961 in experiments using comparable methods in animal systems. When given in equivalent dosage of 25 mg/kg intraperitoneally in mice, plasma interferon levels were 10-fold higher after CP-28,888-27 administration than after CP-20,961 administration. Not only was interferon stimulation superior with CP-28,888-27 administration than with CP-20,961 administration in mice, but also protection against challenge with vaccinia and encephalomyocarditis viruses was superior with the newer compound. In hamsters, reduction of symptoms due to influenza A virus infection was demonstrated with CP-28,888-27 but not with CP-20,961. Also, interferon stimulation in plasma of rats was demonstrated with CP-28,888-27 but not with CP-20,961. In spite of these in vivo effects, neither CP-20,961 nor CP-28,888-27 stimulates interferon in cell cultures, nor do they confer protection of tissue culture cells against challenge with vesicular stomatitis virus. However, both compounds enhance the protective activity of polyinosinic acid:polycytidylic acid in such cultures, and they are equally potent in this regard.

In our study, neither a clinical effect nor an effect on virus shedding was observed. Interesting, in view of the animal data, were the low titers of nasal wash interferon detected, all specimens containing between 5 and 10 IU. In two prior studies with CP-20,961, a total of 19 of 25 subjects infected with a respiratory virus developed interferon in nasal washes, and titers ranged as high as 2,560 IU with a mean of 1.287 (1, 2). It seems likely that the failure to observe a clinical effect may be related to the failure of CP-28,888-27 to induce large quantities of interferon. Since the stock of CP-28,888-27 used for the present studies had also been used in the animal systems described above, it seems unlikely that the lot of drug used was faulty. Rather, it appears that in humans, CP-28,888-27 is a less potent interferon inducer than CP-20,961, at least when administered intranasally.

These studies emphasize the significance of species variation in response to interferon inducers. It is apparent that such compounds must be tested in humans to determine potential usefulness. The concept of use of interferon or interferon inducers to control or alleviate viral upper respiratory illness remains valid.

**LITERATURE CITED**


