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Efficacy of Oral WIN 54954 for Prophylaxis of Experimental Rhinovirus Infection

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The efficacy of oral WIN 54954 for the prevention of rhinovirus infection and illness was tested in two randomized, double-blinded, placebo-controlled volunteer challenge studies. Volunteers were inoculated with rhinovirus type 39 (MIC of WIN 54954, 0.17 μg/ml) or rhinovirus type 23 (MIC, 0.016 μg/ml). The volunteers received two doses of drug (600 mg per dose) or placebo on the first day; this was followed by three doses on each of the subsequent 5 days. All volunteers were challenged with virus after the third dose of study drug. No significant antiviral or clinical effect was detected in either study. Pharmacokinetic studies revealed that on the last day of drug administration, 38 of 39 (97%) volunteers had trough levels of WIN 54954 in plasma greater than the MIC for the respective virus. Nasal wash specimens collected on the same day revealed a detectable level in only 6 of 24 (25%) volunteers at the peak (range, 6 to 24 ng/ml) and in only 2 of 14 (14%) volunteers at the trough (range, 6 and 7 ng/ml). These results suggest that the lack of efficacy of WIN 54954 against rhinovirus may be related to an inability to deliver sufficient drug to the site of viral infection.

The oxazolines are a class of antipicornavirus compounds which have activity against rhinoviruses and enteroviruses in vitro (4, 6, 16) and against enteroviruses in animal models (4, 5). These compounds bind to a hydrophobic pocket in the picornavirus viral capsid protein (7). Depending on the virus serotype, this interaction inhibits replication either by interfering with uncoating of the virus or by causing a conformational change in the cell receptor site on the viral capsid and inhibiting attachment to the cell (9, 14). WIN 54954 is a representative of the oxazoline compounds and has a broad spectrum of activity against both the human rhinoviruses and enteroviruses in vitro (16). In cell culture, this agent inhibited 80% of 52 rhinovirus serotypes when it was used at a concentration of 0.28 μg/ml and 80% of 15 enterovirus serotypes when it was used at a concentration of 0.06 μg/ml. The purpose of these two parallel studies done at the University of Virginia (UVa) and at the Medical University of South Carolina (MUSC) was to determine the efficacy of oral WIN 54954 for the prevention of experimentally induced rhinovirus infection and illness.

MATERIALS AND METHODS

Study design. The clinical trials at both study sites were randomized, double blind, and placebo controlled in design. All volunteers were begun on the study drug or placebo approximately 18 h prior to virus challenge. At UVa the volunteers were isolated in individual motel rooms throughout the treatment period; at MUSC the volunteers were not isolated during the study. The study at UVa was done in January 1990, and the study at MUSC was done in November 1990.

Volunteers. Healthy volunteers between 18 and 55 years of age who had a serum neutralizing antibody titer to the study virus of less than 1:8 were selected for participation in the studies. Volunteers who had an upper respiratory infection or who had taken any medications in the week prior to test drug administration were excluded from the study. Written informed consent was obtained from all study participants. These studies were approved by the institutional review boards at UVa and MUSC.

Study drug. WIN 54954 was supplied as 100- and 400-mg capsules. Each volunteer was given 600 mg per dose, two 100-mg capsules and one 400-mg capsule, or matching placebos every 8 h for 6 days beginning on the afternoon of the first study day. Dosing was continued every 8 h for a total of 17 doses. The dose of drug selected for these studies was based on phase I pharmacokinetic data which indicated that a 600-mg dose consistently results in levels in plasma greater than the MIC for the study viruses and that higher oral doses of drug do not result in higher concentrations in plasma.

Virus challenge. All volunteers were challenged with safety-tested pools of rhinovirus 2 h after administration of the third dose of the study drug on the morning of the second study day. At UVa volunteers were challenged with rhinovirus type 39 (RV39), and at MUSC volunteers were challenged with rhinovirus type 23 (RV23). The MICs of WIN 54954 were 0.17 μg/ml for the RV39 strain used at UVa and 0.016 μg/ml for the RV23 strain used at MUSC, as determined by a plaque reduction assay (16). The virus inoculum pools were diluted and administered to all volunteers in intranasal drops (0.25 ml per nostril). At UVa subjects were challenged 2 times (the second challenge was given after a 30-min interval) with approximately 400 50% tissue culture infective doses ($TCID_{50}$s) of rhinovirus type 39, and at MUSC subjects were challenged with approximately 300 $TCID_{50}$s of rhinovirus type 23.

Measures of infection. Specimens for culture of virus were obtained daily on study days 1 to 10 by nasal wash. Each nostril was rinsed with 5 ml of sterile saline, and the collected fluid was mixed 3:1 (vol/vol) with 4× concentrated viral collecting broth containing fetal calf serum and antibiotics. After incubation at room temperature for at least 30 min, 0.2 ml of the sample was inoculated into each of two

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TABLE 1. Effect of oral WIN 54954 on experimental rhinovirus infection and illness

Virus	Study group	No. of volunteers	No. (%)			Days of shedding (%)[b]	No. (%) ill	Mean total symptom score	Mean nasal mucus wt
			Viral shedding	Antibody responses	Total infected[a]				
RV39	Active	13	13 (100)	10 (77)	13 (100)	51	10 (77)	8 ± 6	13.6 ± 12.7
	Placebo	12	12 (100)	10 (83)	12 (100)	60	6 (50)	5 ± 5	11.8 ± 13.4
RV23	Active	22	17 (78)	18 (82)	18 (82)	60	3 (14)	4 ± 5	4.3 ± 7.4
	Placebo	23	21 (91)	18 (74)	22 (96)	51	8 (35)	10 ± 13	9.7 ± 18.8

[a] Defined as presence of virus shedding and/or seroconversion.
[b] Days of virus shedding/total days of virus culture.

tube cultures of human embryonic lung fibroblasts (WI-38 or MRC-5) maintained in Eagle's minimal essential medium containing 2% fetal calf serum. All cultures were incubated in roller drums at 33°C and were examined every other day for the development of a typical rhinovirus cytopathic effect. One isolate from each volunteer who shed virus was neutralized with specific antibody to the challenge virus to confirm the identity of the viral isolate.

Quantitative viral cultures were done on the nasal wash-viral collecting broth mixture for all virus-positive specimens. Serial 10-fold dilutions of these specimens were inoculated onto cultures of human diploid fibroblast cells and were examined for a viral cytopathic effect after 10 days of incubation. For calculation of mean virus titers, specimens which were positive on initial isolation and negative on reisolation for quantitative culture were defined as containing $10^{0.5}$ $TCID_{50}$s/ml, and cultures which were negative on initial isolation were assigned a value of $10^{-0.5}$ $TCID_{50}$s/ml.

Sera collected before and approximately 3 weeks after virus challenge were tested for the presence of neutralizing antibody to the challenge virus by standard methods. Volunteers who had at least a fourfold increase in antibody titer or who shed virus on any study day after challenge were considered infected.

Measures of illness. Symptom scores were recorded twice each day on the 4 days after virus challenge (study days 3 to 6) and once on the morning of study day 7. A symptom score of 0 to 3, corresponding to absent, mild, moderate, or severe symptoms, respectively, was assigned to the symptoms of rhinorrhea, nasal stuffiness, cough, sneeze, sore throat, headache, malaise, and chilliness. The number of colds in each group was determined by adding the highest scores reported for each symptom on study days 3 to 7. Volunteers who had a symptom score of at least 6 over the 5 days of observation and who had either at least 3 days of rhinorrhea or the subjective impression that they had a cold were considered ill. For calculation of the mean total symptom score, the symptoms reported in the morning and the evening on study days 3 to 6 were averaged to provide the daily symptom score. The daily scores from study days 3 to 7 were then summed to provide the total symptom score for each volunteer. The symptoms included in the calculation of the mean total symptom scores for each group were those listed above as well as fever, myalgia, and hoarseness.

Daily nasal mucus weights were determined by providing the volunteers with preweighed nasal tissues for use during the study. Used tissues were stored in airtight containers until they were counted and weighed.

Complete physical examinations, electrocardiograms, and screening laboratory studies were done on all volunteers before and after the treatment period to detect unexpected adverse effects of study drug administration. All volunteers were questioned daily during the course of the study about any systemic symptoms or other problems with drug tolerance.

Pharmacokinetic studies. Limited pharmacokinetic studies were done at both study sites. At UVa, plasma was obtained for determination of the drug concentration at 0 (predose), 2, 4, and 8 h in relation to the first dose of drug on study day 1 and to the afternoon dose of drug on study day 6 (dose 16). Plasma was also obtained before and 8 h after the afternoon dose of drug on study day 4 (dose 10). A nasal wash was obtained 8 h after administration of each morning dose of drug on study days 2 to 7. At MUSC, concentrations of drug in plasma and saliva were determined prior to and 2 h after the afternoon doses of drug on study days 4 and 6 (doses 10 and 16, respectively). Concentrations of drug in nasal wash were determined 2 h after these two doses.

Drug concentrations were measured in the Department of Drug Metabolism at Sterling Winthrop Inc. The lower limit of detection for the assay was 5 ng/ml in nasal wash and saliva samples and 20 ng/ml in plasma samples.

Statistical analysis. Proportions were compared by a two-sided Fisher exact test. Symptom scores and nasal mucus weights were compared by a two-sided Mann-Whitney U test. P values of less than 0.05 were considered statistically significant. All statistical analyses were performed by using commercial software (NWA Statpak; Northwest Analytical, Inc., Portland, Oreg.).

RESULTS

Subjects. At UVa 27 subjects were enrolled in the study. Two subjects, one in the active treatment group and one in the placebo group, were retrospectively found to have a titer of acute-phase antibody to the challenge virus of ≥1:8 and were excluded from the analysis of drug efficacy. At MUSC 53 subjects were enrolled in the study. A total of eight subjects, five in the active treatment group and three in the placebo group, were excluded from the efficacy analysis. The subjects excluded were one in the active treatment group and three in the placebo group who were retrospectively found to have titers of acute-phase antibody to the challenge virus of ≥1:8, two in the active treatment group from whom enterovirus was isolated from the prechallenge virus culture, and two subjects in the active treatment group who failed to comply with the study protocol.

Occurrence of infection and illness. The antiviral effect of the study drug was determined by assessing the proportion of volunteers infected and the quantity of virus shed by the infected volunteers (Table 1). All of the RV39 virus-challenged subjects were infected, and no significant differences

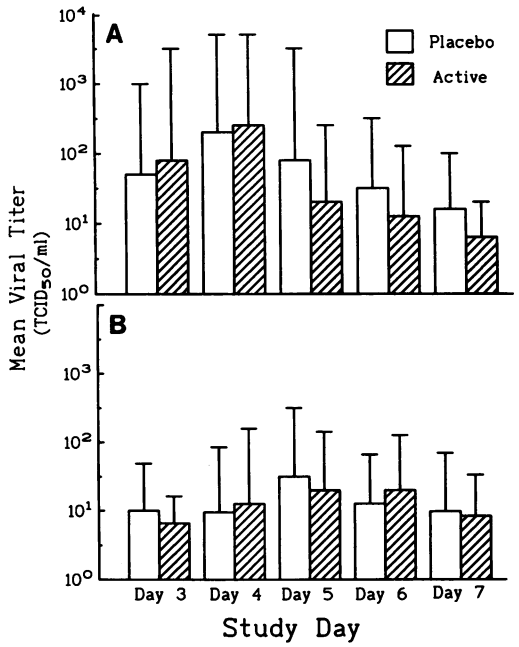

FIG. 1. Geometric mean virus titers in treated and placebo groups in rhinovirus type 39-challenged volunteers at UVa (A) and rhinovirus type 23-challenged volunteers at MUSC (B). Volunteers were challenged with virus on study day 2.

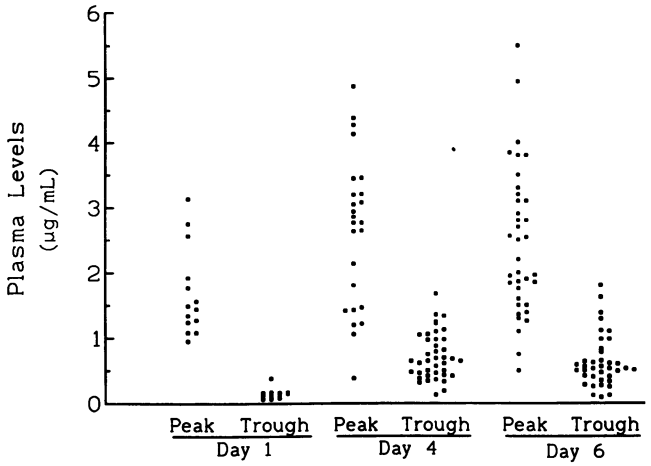

FIG. 2. Peak (2 h after last dose) and trough (8 h after last dose) levels of WIN 54954 in the plasma of human volunteers given 600 mg of the drug every 8 h by mouth.

in the geometric mean titer of virus shed were detected between the treated and placebo groups (Fig. 1). Virus shedding was first detected 2.6 ± 2.7 (mean ± standard deviation) days after challenge in the treated group and 1.5 ± 1.4 days after challenge in the placebo group.

The MIC of WIN 54954 for the RV23 virus used at MUSC was 10-fold lower than the MIC for the virus used at UVa. Despite the increased susceptibility of the virus to the study drug, there were no differences either in the proportion of volunteers who were infected or shed virus (Table 1) or in the geometric mean titers of virus shed in the treated and placebo groups (Fig. 1). Virus shedding was first detected 1.8 ± 1.3 and 1.7 ± 1.5 days after challenge in the treated and placebo groups, respectively.

The clinical effect of the study drug was assessed in the studies described here by comparing symptom scores, the proportion of subjects who developed colds, and nasal mucus weights in the two study groups. No statistically significant differences in any of these parameters were found at either study site (Table 1). Reanalysis of the data to include only those subjects whose acute-phase antibody titer to the challenge virus was ≤1:2 did not alter the outcome of the efficacy analysis.

Pharmacokinetic studies. There was considerable interindividual variability in the concentration of the study drug in plasma (Fig. 2). The mean peak concentrations in plasma on days 1, 4, and 6 of treatment were 1.61, 2.64, and 2.45 μg/ml, respectively. The mean trough levels in plasma on these days were 0.14, 0.73, and 0.64 μg/ml, respectively. On day 6, the trough level of drug in plasma was greater than the MIC for the respective challenge virus for 38 of 39 (97%) treated volunteers.

Despite the levels achieved in plasma by oral administration of 600 mg of WIN 54954, the drug was generally undetectable in nasal washes. Only 10 of 50 (20%) nasal wash specimens collected at MUSC 2 h after drug administration on days 4 and 6 had a detectable level of drug. Three (6%) of the 50 specimens had a drug concentration greater than the MIC for the challenge virus. At UVa only 3 of 42 (7%) nasal wash specimens collected at the trough of the level in plasma were found to have detectable drug. None of the trough levels in nasal wash was greater than the MIC for RV39. At MUSC the mean concentration of study drug in saliva was 12.5 ng/ml at the trough and 22.5 ng/ml 2 h after the dose. The concentration of study drug in saliva exceeded the MIC for RV23 in 13 of 51 (25%) of the specimens collected at the trough and 24 of 50 (48%) of the specimens collected at the peak. The levels in saliva ranged from 0.4 to 7.8% (mean, 1.9%) of the corresponding levels in plasma at the trough and from 0.2 to 6.3% (mean, 1.1%) of the levels in plasma 2 h after the dose.

Tolerance. The study drug was well tolerated at the dose used in the present studies. Four of the 41 (10%) study drug recipients developed a macular rash after discontinuing the study drug. There were no other significant clinical or laboratory abnormalities seen in the study group.

DISCUSSION

Oral administration of WIN 54954 at doses that resulted in substantial concentrations of drug in plasma was ineffective for the prevention of experimental rhinovirus colds in the two challenge studies described here. The lack of efficacy of WIN 54954 was disappointing in view of this agent's excellent in vitro activity against the rhinovirus serotypes studied.

The failure of WIN 54954 in the present trials may be related to several factors. The most likely explanation for the lack of efficacy was the apparent inadequate concentration of drug delivered to the site of infection in the nasal epithelium. The strength of this conclusion is mitigated by the fact that the samples used to determine the drug concentrations in the nasal epithelium were not obtained directly and the concentrations detected in the nasal wash were diluted by the wash procedure. The saliva samples collected in the study at MUSC were obtained in an attempt to address the problem of dilution, and in fact, the levels in saliva were substantially greater than the levels in nasal wash. The levels in saliva, however, were only 1 to 2% of the corresponding levels in plasma. Drug concentrations in both saliva and

nasal wash were below the MIC for the challenge virus in a substantial proportion of treated subjects.

A second potential explanation for the lack of efficacy in the present studies was insufficient experimental power. The largest of these two studies had only a 25% chance of detecting a true reduction of 50% in the incidence of colds and only a 35% chance of detecting a true reduction of 50% in the mean symptom score at a significance level of $P < 0.05$. The limited power of the study to detect an effect on illness was in part due to the unexpectedly low illness attack rate in the placebo subjects. Despite the limited power of the study to detect illness, the study had a 95% chance of detecting a 50% reduction in the incidence of rhinovirus infection. No effect of treatment was seen on either infection rate or virus titer in either of the studies. It is likely that this result is due to a failure of the study drug at the dosage given.

A final possibility to be considered in the analysis of the results of the present study is the potential for development of drug resistance by the challenge viruses. The development of resistant mutants during treatment of human volunteers with capsid-binding agents has been reported (3). Furthermore, mutants of human rhinoviruses which result in high-level resistance to the oxazoline compounds have been observed (12). Forty-eight viral isolates from the UVa trial reported here were tested for their susceptibilities to the study drug, and no resistant mutants were found (13). The similarities in the proportion of study days with a virus isolate and the median time to isolation of virus in the treated and control groups also suggest that development of resistance is an unlikely explanation for our results.

Our results are similar to the results of clinical trials involving other chemically distinct capsid-binding antirhinoviral agents. With the exception of the pyridazinamine class of drugs, these agents have generally been found to be ineffective in vivo. The chalcones, which have potent in vitro activity against the rhinoviruses, were ineffective whether given orally or intranasally (2, 15). The pyridazinamines R61837 and R77975 or pirodavir have been shown to provide partial protection against experimentally induced illness when given by frequent intranasal sprayings (six times per day); however, breakthrough illness occurred after cessation of spraying and less frequent spraying (three times per day) was ineffective (1, 11). The experience with alpha interferon demonstrates that effective antiviral therapy delivered to the site of infection prevents rhinovirus infection and illness (8, 10). The potential for clinical efficacy of the oxazoline compounds appears to depend on the development of drugs with increased potency or formulations that can be delivered effectively to the nasal mucosa.

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