Clinical Activity of Pleconaril in an Experimentally Induced Coxsackievirus A21 Respiratory Infection

Gilbert M. Schiff and James R. Sherwood

A randomized, double-blind study assessed the efficacy and safety of pleconaril, a novel antiviral drug with broad-spectrum activity against picornaviruses, in the treatment of 33 adults with an experimentally induced viral respiratory infection. Subjects received either pleconaril 200 mg twice daily (initial dose of 400 mg) or placebo for 7 days. Fourteen hours after receiving the initial dose of either pleconaril or placebo, subjects were inoculated intranasally with 100 plaque-forming units of coxsackievirus A21. Results revealed statistically significant reductions in viral shedding in nasal secretions (P < .001), nasal mucus production (P = .004), and total respiratory illness symptom scores (P = .013) in pleconaril-treated as compared with placebo-treated subjects. The most common adverse events were nausea and abdominal pain. These data support the safety and efficacy of pleconaril in decreasing the signs and symptoms and viral shedding associated with a viral respiratory infection.

Respiratory tract infections, which are largely caused by viruses, have a major impact on patient morbidity and healthcare use. According to recent data, >30 million office and emergency department visits occur annually, secondary to acute respiratory tract infections [1]. Although the mortality due to viral respiratory infection is rare in developed countries, viruses play a contributing role in approximately 20%–30% of the 4.5 million respiratory tract infection–related deaths that occur annually among children aged <5 years residing in developing countries [2, 3].

The majority of viral diseases are caused by the Picornaviridae, a family of small single-stranded RNA viruses [4]. Rhinoviruses, which constitute the largest genus in the Picornaviridae family [5], have been implicated in one-third to one-half of all cases of acute respiratory disease and represent the single most important pathogen in the common cold [6]. The enterovirus genus of the Picornaviridae family includes 72 strains (poliovirus and several nonpolio) of enteroviruses. Although poliovirus is largely contained, as a result of global eradication programs [7, 8], the nonpolio enteroviruses are responsible for approximately 15% of upper respiratory tract infections in which a pathogen is identified [9]. Patients infected with rhinovirus or enterovirus typically present with low-grade fever and nonspecific respiratory tract symptoms such as cough and increased nasal secretions.

Pleconaril is an orally active, low-molecular-weight agent that integrates into hydrophobic pockets within the capsid protein VP1, resulting in increased virion stability and inhibition of virion attachment and uncoating [10]. Pleconaril has potent and broad-spectrum antipicornaviral activity, as demonstrated by in vitro [11, 12] and clinical [13, 14] testing. The current study assessed the efficacy and safety of repeated doses of pleconaril in reducing viral shedding and relieving the symptoms and severity of clinical illness in adult subjects with an experimentally induced viral respiratory infection.

Eligible subjects were randomized on a 1:1 basis to receive either oral pleconaril, at an initial dose of 400 mg followed by 200 mg twice daily for 7 days, or matching placebo. Doses of pleconaril or placebo were administered at 0700 and 1900 h (± 0.5 h) with approximately 120 mL of water. Hand and mouth checks were performed to ensure ingestion of the study medication.

The first dose of pleconaril (400 mg) or placebo was administered at approximately 1900 hours on study day 1. After administration of the second dose of pleconaril (200 mg) or placebo (14 h after the initial dose), subjects were inoculated intranasally with 100 plaque-forming units (pfu) of coxsackievirus A21. Previous reports of intranasal instillation of 100 pfu of an attenuated strain of coxsackievirus A21 in healthy volunteers describe the production of a robust coldlike illness with systemic symptoms and signs in approximately 60%–70% of inoculated subjects [15, 16, G. M. Schiff, unpublished data].
Subjects and Methods

Study population. Healthy nonsmoking men and women aged 18–55 years were eligible to participate. Subjects underwent a complete medical history evaluation, physical examination (including vital sign evaluation and clinical laboratory tests), and a brief neurologic examination to determine study eligibility.

Eligible subjects were required to be without detectable serum neutralizing antibodies to coxsackievirus A21 (defined as a serum neutralizing antibody titer ≤1 : 4). Eligible women were not pregnant or nursing and either were surgically sterile or were using an effective form of contraceptive. The following subjects were excluded: those with a history or presence of any significant disease state, including allergic skin or respiratory disease; those having a history of upper respiratory tract infection, fever within 1 week of the study or exposure to an upper respiratory tract infection within 3 days of study onset; human immunodeficiency virus (HIV)-positive individuals; and those with a history of drug or alcohol abuse. Subjects who had ingested any nonprescription or prescription drugs (excluding oral contraceptives) during the preceding week, had donated ≥1 pint of blood within 4 weeks prior to the initial administration of study drug, or had received any other investigational drug within 30 days of the study were excluded.

Study methods. This double-blind, parallel-group, single-site study was approved by the institutional review board, and written informed consent was obtained from each eligible subject prior to initiation of any study procedures. The study was performed in the United States between 10 February 1996 and 18 March 1996.

All subjects were confined to the study unit beginning on the morning of study day 1 (the first day of dosing) and continuing through the morning of study day 8. Subjects consumed a standard breakfast or dinner meal within 1 h (± 0.25 h) prior to dosing. Alcohol consumption was not permitted during the study unit confinement period. Acetaminophen ingestion was allowed for fever >39°C, and propoxyphene was allowed for severe pain.

At the time of discharge, subjects were told to return to the study unit as outpatients on study days 8, 9, and 10, for further clinical and virologic assessments, and at approximately study day 28 after viral challenge, for serologic evaluation. Subjects were also told to return to the study unit for further evaluations if symptoms occurred beyond study day 10. Those who remained symptomatic were kept at the study site as long as deemed necessary by the study investigator, to ensure the subjects’ health and safety.

Viral inoculation. At 0900 h on study day 2 (approximately 2 h after the morning dose) each subject received an intranasal inoculation of 100 pfu of coxsackievirus A21 suspended in 0.5 mL of Earle’s balanced salt solution. The inoculum was administered by a slow intranasal drip, with approximately 0.25 mL instilled in each nostril by a 1-mL graduated pipette. Subjects remained in a supine position with the neck hyperextended for approximately 20 min after instillation.

The strain of coxsackievirus A21 (lot 48654), obtained from Dr. Robert Couch (Baylor College of Medicine, Houston), had been repeatedly passaged in Wistar-26 (diploid human embryonic lung cells), partially purified, and stored at −70°C for approximately 25 years. Aliquots had been safety tested according to the procedure of Knight [17]. An additional passage was made in MRC-5 (diploid human embryonic lung cells) and Wistar-26 cells, harvested and combined, and centrifuged, and the supernatant was frozen. Later, the frozen pool was purified by high-speed and glycerol-gradient centrifugation. The purified virus in 20% glycerol was diluted 2-fold in 0.75-mL aliquots to produce a titer of 6 × 10⁴ pfu as measured in MRC-5 cells. Aliquots were safety tested to verify the absence of microbial (bacterial, viral, fungal, or mycoplasma) adventitious agents by means of standard assays.

Viral shedding in nasal wash. Nasal-wash specimens were collected on study day 1 (prior to inoculation) and once daily on study days 2–10, to assess viral shedding. Specimens were obtained by tilting the subject’s head back and gently instilling approximately 5 mL of lactated Ringer’s solution into each nostril by use of a 10- to 20-mL syringe. Each subject held the solution in the nostril for a brief period and then forcibly expelled it into a labeled collection container. The procedure was repeated in the opposite nostril and the solution expelled into the same-labeled collection container. Specimens were held on ice for <2 h until processed. Each specimen was homogenized by repeated forcing through a syringe, stabilized with the addition of one-seventh volume of a 7% solution of veal infusion broth, and frozen at −70°C until assayed. At the time of assay, quadruplicate 10-fold dilutions of each specimen were prepared in Eagle’s minimum essential medium (EMEM) with 10% tryptose phosphate broth and inoculated onto confluent monolayers of MRC-5 cells in 96-well plates. The plates were incubated at 37°C and were examined for cytopathic effect at 7 days. Titers were calculated according to the method of Reed and Muench [18]. The initial isolate from each subject was identified as coxsackievirus A21 by neutralization with specific antisera purchased from ATCC (Manassas, VA).

Antibody titers. Blood specimens for coxsackievirus A21–neutralizing antibody titer assay were collected at subject screening, prior to the first dose of test drug on study day 1, and at approximately 4 weeks after viral challenge, to determine baseline antibody activity and development of antibody response to the exposure to virus. A volume of approximately 10 mL of blood was collected in nonheparinized tubes for each sample and was allowed to clot. Samples were centrifuged at 4°C for 10 min at 1500 g. The serum was removed by pipette, aliquoted into 4 polypropylene tubes, and stored at −20°C. Serum-neutralizing antibody titers were determined by preparing 2-fold dilutions of serum in EBSS + 10% TBP, mixing with 100 pfu coxsackievirus A21, and inoculating onto confluent monolayers of MRC-5 cells in 60-mm dishes. Plates were overlaid with EMEM with 0.2% agarose, 5% fetal bovine serum, and antibiotics. After 3 days of incubation at 37°C in an atmosphere of 5% CO₂, the overlay was removed, the monolayers stained with crystal violet solution, and the plaques counted. The serum neutralization titer was defined as the reciprocal of the highest dilution that reduced the plaque count 60% relative to a serum-free viral control.

Nasal mucus production. Nasal mucus weights were determined daily until discharge from the study unit on study day 8. Facial tissues used by subjects were immediately stored by the subjects in zip-lock plastic bags. Plastic bags were weighed twice daily (at approximate 12-h intervals). The bag containing the tissues was weighed (gross weight), the tissues counted, and the weight of the bag and tissues subtracted from the gross weight, to determine nasal mucus weight. The total mucus produced daily by each subject was calculated.
Subjective respiratory symptom and global illness assessment.

Subjective rating of respiratory illness symptoms and global assessment of respiratory illness were performed once on study day 1, twice on study days 2–7, and once daily on study days 8–10. Subjects rated their respiratory illness symptoms by grading each of 10 specific dimensions on a scale of 0–3 where 0 indicated no symptoms; 1, mild symptoms; 2, moderate symptoms; and 3, severe symptoms. The 10 specific dimensions were grouped into 3 categories: respiratory signs and symptoms including rhinorrhea (runny nose), nasal stuffiness, cough with pain, and sore throat; systemic signs and symptoms including headache, malaise, myalgia, feverishness (fever scores of 1, 2, and 3 were assigned to temperatures of 39°C to 39.5°C, >39.5°C to 40°C, and >40°C, respectively), and chills/sweat; and other respiratory or systemic signs and symptoms not listed above. A total respiratory illness symptom score of ≥5 and <10 was considered mild illness, and a score of ≥10 was considered moderate illness. The global assessment of respiratory illness was performed by subjects rating the overall severity of their respiratory illness using a 0–10 analogue scale in which 0 indicated no illness and 10 indicated severe illness.

Pleconaril plasma concentration.

Blood samples (3 mL) for analysis of pleconaril plasma concentration were obtained from each subject immediately before and 2 h after the first dose of study medication on study day 1; immediately before the morning dose on study days 2 and 7; and at 2, 4, and 12 h after the morning dose on study days 2 and 7. The 12-h blood samples were collected immediately prior to administration of the evening dose on study days 2 and 7. Collection tubes contained ethylene/diamine/tetracetic acid. Pleconaril concentrations were determined at Phoenix International (Montreal) by use of gas chromatography with electron capture detection. The limit of detection for the assay was established as 49.4 ng/mL. The analytical method demonstrated linearity at plasma concentrations ranging from 49.4 to 1975.7 ng/mL. Interday assay variability was consistently <10.2% for concentrations within the range of linearity.

Safety evaluation.

The safety of pleconaril was evaluated by continuous surveillance for adverse events and by assessment of vital signs (including blood pressure and heart rate after sitting for at least 5 min, sitting respiratory rate, and oral temperature), physical examination, neurological examinations (including attention span, gait, balance and coordination, deep tendon reflexes, and muscle strength), and clinical laboratory evaluations (including hematology, baseline chemistry, and urinalysis). Oral temperature was measured every 8 h during the study confinement period. If fever (i.e., temperature >39°C) occurred, temperature was measured every 2 h until it returned to normal. Subjects with temperatures that exceeded 39°C (102.2°F) were treated with acetaminophen.

Statistical analysis.

This study was powered (1–β = 0.80) to detect a reduction in illness attack rate of 50% by using a two-sided χ² test at a significance level (α) of .05. The resultant sample size assumed a 90% incidence of infection in each group and at least a 67% incidence of symptomatic illness among infected individuals in the placebo group. A repeated-measure analysis of variance (ANOVA), including treatment group and day and both within- and between-subject error terms, was used for determination of an overall treatment effect. If the overall test was significant (α = 0.05), individual t tests were used to test treatment effect for each observation day. Statistical analyses were performed with SAS Version 6.12 (SAS Institute, Raleigh, NC).

Baseline subject demographic and medical characteristics were summarized by treatment group by use of descriptive statistics. Daily mean viral titters were compared between treatment groups.
on each study day by use of ANOVA methods. Fisher’s exact test was used to compare the proportions of subjects in each treatment group with illness (defined as peak mucus weight >5 g or peak respiratory illness score >5), the distribution of subjects within the nasal mucus weight and respiratory illness symptom score categories (peak nasal mucus weights of 0–5, >5 and ≤10, >10 and ≤15, or >15 and peak respiratory illness symptom scores were of 0–5, 6–10, 11–15, or 16–36), the distribution of subjects within global assessment score levels (0–3, 4–5, 6–7, and 8–10), and maximum oral temperatures (<37.8°C, 37.8°C to 38.3°C, 38.4°C to 38.9°C, and >38.9°C). The number of subjects who seroconverted during the study (defined as a 4-fold increase in titer) was tabulated to determine the incidence of infection. Pleconaril plasma concentration data were summarized by use of descriptive statistics. Treatment-related adverse events were grouped by body system by use of the COSTART dictionary and were tabulated according to incidence and severity. Clinical laboratory and vital-sign parameters were evaluated for change (mean and median) from baseline to the end of treatment.

Results

Subject population. A total of 33 adult volunteers were randomized to receive pleconaril (16 subjects) or placebo (17 subjects). One subject randomized to the placebo group experienced symptoms of an upper respiratory infection during the baseline evaluation period; however, he was included in all analyses. One subject with a neutralization titer >1:4 at screening was included in all analyses of efficacy and safety with the exception of infection rate. The 2 treatment groups were similar with respect to demographic characteristics, baseline respiratory rate, and oral temperature (table 1). Concomitant medications were taken by 13 (76%) placebo-treated subjects (acetaminophen, 11; propoxyphene, 7; cough/sinus preparations, 6) and 9 (56%) pleconaril-treated subjects (acetaminophen, 9) during the study.

Viral shedding in nasal secretions. Subjects treated with pleconaril exhibited significantly lower geometric mean viral titers on study days 3 and 4 (P < .001) and study day 7 (P < .05), compared with placebo-treated subjects (figure 1).

Antibody titers. Of the 31 subjects who had a neutralization titer ≤1:4 at baseline, 29 (94%) had seroconverted (as indicated by at least a 4-fold increase in serum neutralizing antibody titer) by study day 28. Two subjects randomized to pleconaril did not seroconvert.

Nasal mucus production. Peak mean nasal mucus production was consistently lower in pleconaril-treated subjects as compared with placebo-treated subjects. After viral inoculation, a peak mean daily mucus production of 7.3 g occurred on study day 5 in placebo-treated subjects as compared with a peak mean daily mucus production of 3.0 g that occurred on study day 3 in pleconaril-treated subjects. The volume of nasal mucus produced by placebo-treated subjects was significantly (P = .016) greater than that produced by pleconaril-treated subjects (figure 2). Placebo-treated subjects exhibited a significantly higher frequency of illness, as defined by the percentage of subjects with nasal mucus production of >5 g at any time, as compared with pleconaril-treated subjects (65% [11/17] vs. 13% [2/16]; P = .004).

Respiratory symptom scores and global assessment of illness. Pleconaril-treated subjects exhibited significantly (P = .015) lower total respiratory symptom scores, when stratified by total respiratory symptom score categories, as compared with placebo-treated subjects (figure 3). Subjective assessments revealed a significantly higher frequency of illness, as defined by the percentage of subjects with a respiratory illness symptom score of >5 at any time, in placebo-treated subjects as compared with pleconaril-treated subjects (65% [11/17] vs. 19% [3/16]; P = .013). A trend was evident in the evaluation of subjects’ global assessment of illness, with subjects treated with pleconaril reporting less-severe disease as compared with placebo-treated subjects. Subjects treated with pleconaril also tended to have lower peak oral temperatures as compared with placebo-treated subjects.

Plasma concentrations of pleconaril. On study day 2, mean plasma levels of pleconaril at 2 and 4 h postingestion were 874 ± 469 ng/mL and 1044 ± 307 ng/mL, respectively, among the subjects given pleconaril. Mean plasma levels of pleconaril on study day 7 at 2 and 4 h postdose were 1139 ± 362 ng/mL and 1199 ± 241 ng/mL, respectively. The minimum plasma pleconaril concentrations (i.e., trough concentrations) obtained on study days 2 and 7 (440 ng/mL and 521 ng/mL) were several times higher than the concentration needed to inhibit 50% (IC50) of isolates (40 ng/mL) of the safety-tested strain of coxsackievirus A21 used in the study.

Safety assessment. The incidences of adverse events in the pleconaril and placebo groups were similar. All reported events were mild in intensity and resolved spontaneously. No subject

<table>
<thead>
<tr>
<th>Table 1. Baseline demographic and clinical characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>Weight (kg), male</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>Weight (kg), female</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Race</td>
</tr>
<tr>
<td>Black</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>Respiratory rate (rpm)</td>
</tr>
<tr>
<td>Range</td>
</tr>
<tr>
<td>Oral temperature (°C)</td>
</tr>
<tr>
<td>Range</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) except as indicated.
discontinued the study prematurely because of an adverse event and no serious adverse events or deaths were reported.

Eight (47%) placebo-treated subjects reported 19 adverse events and 8 (50%) pleconaril-treated subjects reported 12 adverse events. Nausea was the most frequently reported adverse event, occurring in 3 (18%) placebo-treated and 4 (25%) pleconaril-treated subjects. Abdominal pain occurred in 3 (18%) placebo-treated and 1 (6%) pleconaril-treated subject. Of all reported events, 11 unique events were considered to be related to study drug administration by the investigator: 6 occurred in placebo-treated subjects (nausea, 2; abdominal pain, 2; dyspepsia, 1, and amblyopia, 1) and 5 occurred in pleconaril-treated subjects (nausea, 2; dysgeusia, 1, dysmenorrhea, 1; and urinary frequency, 1).

No clinically significant adverse trends were noted in laboratory parameters, vital signs, physical examinations, or neurological examinations in pleconaril-treated subjects relative to placebo-treated subjects.

Discussion

The results of this study document that pleconaril, administered at a dose of 200 mg twice daily to adult volunteers, effectively decreases viral replication and reduces the signs and symptoms of an experimentally induced viral respiratory infection. Subjects given pleconaril experienced significant reductions in viral shedding, nasal mucus production, and total respiratory illness symptom scores as compared with placebo-treated cohorts. Higher percentages of placebo-treated subjects experienced illness after inoculation with coxsackievirus A21, as indicated by higher volumes of nasal mucus production (i.e., >5 g) and higher respiratory illness symptom scores (i.e., >5) noted during the study period. More placebo-treated subjects required the use of concomitant medications to relieve the signs and symptoms (i.e., headache, nasal stuffiness, and feverishness) of their viral respiratory infection.

Several potential clinical benefits are associated with the findings of this study. First, a reduction in viral shedding due to pleconaril treatment may result in patients being less likely to transmit their viral infections to others, thereby decreasing the overall incidence of viral respiratory infections. Second, treatment with pleconaril in patients with viral respiratory infection ameliorates the signs and symptoms of illness, thereby resulting in a decrease in days lost from work and/or school. These patients would require decreased use of health care resources, such as medications, physician visits, and emergency department visits, and would reduce the likelihood of exacerbation of other concurrent illnesses (e.g., asthma) by the viral infection.

In this study subjects were inoculated with a safety-tested strain of coxsackievirus A21, which has been found in previous studies to produce a mild febrile respiratory illness with signs and symptoms that closely mimic a naturally occurring viral respiratory infection [19, 20, G. M. Schiff, unpublished data]. The coxsackievirus A21, like the rhinoviruses and other enter-
pleconaril in respiratory infection

Figure 3. Distribution of total respiratory symptom scores, by treatment group

oviruses, causes subjects to experience symptoms of respiratory illness that may persist for weeks [21–24]. It is important to note the limitations of the design used in this trial (drug treatment prior to infection) relative to the clinical scenario of pleconaril treatment of an established viral infection.

Pleconaril is a novel compound whose antiviral mechanism of action may be due to early inhibition of the viral adherence, maturation, and the replication process. It is theorized that pleconaril integrates deep within the hydrophobic pockets of the virus capsid, interrupting viral protein function necessary for viral attachment and uncoating [10]. Interruption of these steps prevents viral RNA release, replication of the virus, and production of progeny virions. As confirmed by the results of this study (i.e., nasal viral shedding and the similar distribution of convalescent antibody titers measured 4 weeks after the coxsackievirus inoculation in the pleconaril and placebo groups), pleconaril decreases the amount of virus in the nasal mucus but does not appear to affect the production of neutralizing (protective) antibodies. Therefore, it appears that pleconaril produces significant virologic effect and clinical benefits without compromising the subject’s ability to mount an immune response against the infection.

The pleconaril plasma concentrations found on study day 2 (874 ± 469 ng/mL and 1044 ± 307 ng/mL at 2 and 4 h after ingestion, respectively) and on study day 7 (1139 ± 362 ng/mL and 1199 ± 241 ng/mL, at 2 and 4 h after ingestion, respectively) are similar to those found in previous investigations. A study of single-dose pleconaril pharmacokinetics in healthy volunteers revealed a mean maximum serum concentration (C_{max}) of 1272 ng/mL and a mean time to maximum serum concentration (T_{max}) of 4.1 h [25]. In a clinical study in which multiple doses were used, the mean maximum C_{max} at steady state of pleconaril administered at a dose of 200 mg twice daily for 7 days was 1330 ng/mL, with trough concentrations ranging between 270 and 540 ng/mL [26]. The concentrations of pleconaril attained in this study after multiple administrations of 200 mg, even at minimum (i.e., trough) plasma levels, were sufficiently high to inhibit viral replication. After 2 doses of pleconaril, trough plasma concentrations (i.e., 351 ng/mL) were found to be 3-fold to that found in prior studies (100 ng/mL), to inhibit >95% of clinical enteroviral isolates [12], and to be well above the median concentration needed to inhibit at least 50% (IC_{50}) of the safety-tested strain of coxsackievirus A21 used in the study.

Pleconaril was safe and well tolerated when given as a 400-mg initial dose followed by 200 mg twice a day for 6.5 days (a total of 13 doses, 2800 mg). No deaths, serious adverse events, or premature discontinuations due to adverse events were reported in this study. The most commonly reported adverse events were nausea and abdominal pain among placebo-treated subjects (3 subjects each) and nausea among pleconaril-treated subjects (4 subjects). The dysmenorrhea and urinary frequency reported most likely represent common complaints among sexually active women.

In conclusion, this study further supports the antiviral efficacy, tolerability, and safety of oral pleconaril in the treatment...
of adults inoculated with coxsackievirus A21, a strain that produces signs and symptoms that closely mimic those of a naturally occurring viral respiratory tract infection. Further investigations with plecanaril in disease states caused by picornaviruses, including viral respiratory tract infections, are warranted.

Acknowledgments

We thank the clinical research nurses of the Gamble Program at Children’s Hospital Medical Center for help on all clinical aspects; Richard Ward for help on virological laboratory assays; and Mark McKinlay for help in arranging for the pharmacokinetic assays.

References

1. National Ambulatory Medical Care Survey. Atlanta, GA: Centers for Disease Control and Prevention, 1996.