Inhaled ICI 204,219 Blocks Antigen-Induced Bronchoconstriction in Subjects With Bronchial Asthma*

Robert A. Nathan, M.D., F.C.C.P.; Mitchell Glass, M.D.; and Margaret C. Minkwitz, Ph.D.

Three inhalation formulations of ICI 204,219 were compared for antagonism of antigen-induced bronchoconstriction in 16 subjects with asthma who demonstrated reproducible hypersensitivity to allergen during screening challenges. Each subject received a single 0.2-mg dose of each formulation and was challenged with ragweed 30 min after administration of ICI 204,219 until the forced expiratory volume in 1 s (FEV₁) decreased by 20 percent or the maximum allergen concentration (100 µg/ml) was reached. The majority of subjects tolerated 100 µg/ml of allergen without a 20 percent decrease in FEV₁. Inhalation formulations of ICI 204,219 successfully inhibited bronchoconstriction in subjects with reproducible sensitivity to ragweed challenges.

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METHODS

Subject Selection and Study Design

Sixteen subjects with asthma, aged 20 to 43 years, were enrolled in this open-label, three-period, crossover study. Subjects who were selected for the study met the American Thoracic Society criteria for asthma. They were nonsmokers, showed no clinical signs or symptoms of airway obstruction, had a forced expiratory volume in 1 s (FEV₁) ≥ 65 percent of the predicted normal value, and demonstrated a reproducible sensitivity to bronchoprovocation with a standardized allergen (giant ragweed, Hollister-Stier, Spokane, Wash.). The FEV₁ before the second screening challenge had to be within ± 5 percent of the first prediluent FEV₁ to show comparable baseline airway function. Reproducible sensitivity was defined as a 20 percent decrease in FEV₁ during both screening challenges at final allergen concentrations within two dilutions (a difference of no more than a factor of four). Subjects were excluded from the study if they had any acute illness or disease, a history of drug or alcohol abuse, an upper or a lower respiratory tract infection, or immunization with live attenuated influenza virus within 6 weeks of the study. Subjects could not use inhaled or oral steroids, cromolyn sodium, or long-acting theophylline for the treatment of asthma. Acetaminophen was the sole nonprescription medication permitted for analgesia. Subjects were not permitted to receive prescription medications for acute exacerbation of asthma within 12 h of each trial period. Prescription medication was permitted during the study only after mutual consent of the investigator and the sponsor. All subjects were prohibited from consuming alcohol, caffeine, or any medication (other than those permitted above) that could theoretically interact with the trial medication. Written informed consent was obtained from each subject, and the study was approved by the appropriate Institutional Review Board (Western Institutional Review Board, Olympia, Wash.).

During the 2 weeks before the trial, each subject provided a medical history and underwent a complete physical examination.

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Antigen-induced Bronchoconstriction in Bronchial Asthma (Nathan, Glass, Minkwitz)

12-lead electrocardiography, routine clinical laboratory tests, a urine screen for drug abuse, pulmonary function tests, measurements of vital signs, and a determination of subjective symptoms. Subjects stopped taking all asthma and allergy medications at least 12 h before entering the study.

The study consisted of three successive treatment periods separated by 3-day to 4-week intervals. Total treatment was completed within 3 months and took place when there was no evidence of ragweed in the environment. Three formulations of ICI 204,219 were used in this trial. Each formulation was a suspension of ICI 204,219 in chlorofluorocarbons combined with a surfactant, which served as a suspending agent and aided in lubricating the valve. Formulation 1 contained an amorphous bulk drug that is used in oral formulations, whereas formulations 2 and 3 contained a crystalline bulk drug. The delivery and dispersal characteristics were not significantly different among the three formulations. A single 0.2-mg dose of each formulation of ICI 204,219 was administered during treatment periods by means of a metered-dose inhaler: formulation 1, delivered in four actuations at 0.05 mg of drug per actuation; formulation 2, delivered in four actuations at 0.05 mg of drug per actuation; and formulation 3, delivered in one actuation at 0.2 mg of drug per actuation.

Bronchoprovocation was performed with a standardized ragweed allergen, provided the subject had an FEV, of at least 65 percent of the predicted normal value and within ± 15 percent of the screening FEV, value. Allergen was delivered by means of a nebulizer (DeVilbiss No. 646, DeVilbiss Company, Somerset, Pa) during slow, submaximal inspiration from functional residual capacity to near total lung capacity. The nebulizer was attached to a dose-monitoring device (Rosenthal-French Dosimeter, Laboratory for Applied Immunology, Inc, Fairfax, Va), consisting of a breath-actuated solenoid valve timing circuit and compressed air supply at 20 pounds per square inch (PSI). The solenoid valve was set to remain in the open position for 0.6 s at the onset of inspiration. After the solenoid valve closed, inspiration continued for another 0.5 s. Each subject was instructed to hold his or her breath for 2 to 5 s after inhalation before exhaling.

Efficacy Assessment

The efficacy of ICI 204,219 was assessed by measuring pulmonary function before and after bronchoprovocation during each treatment period. Following administration of ICI 204,219, subjects inhaled five breaths of control aerosol consisting of saline solution solvent. Baseline FEV, and forced vital capacity (FVC) were measured 10 min later. A dose-response curve was then derived for each subject after administering five inhalations of antigen at each of a series of increasing concentrations, with 10- to 15-min intervals between successive concentrations.

The allergen concentrations used in provocations are presented in Table 1. The initial allergen dose for each subject (for periods 1, 2, and 3) was selected on the basis of results from the screening bronchial challenges. The starting concentration selected was one dilution number higher than the lowest concentration needed to provoke a decrease of 20 percent in FEV, (PC20) during the two screening challenges. Up to 8 doses of antigen were inhaled by each subject during the screening challenges, and up to 12 doses were administered on treatment days. The concentration of successive doses of antigen was doubled repeatedly until the FEV, decreased by at least 20 percent of the value obtained after the administration of the control aerosol, the maximum antigen concentration had been administered (100 μg/ml), or the subject had symptoms that warranted terminating the test. The same sequence of increases in antigen doses was repeated with each ragweed challenge performed on a particular subject. The FEV, and FVC were measured at 10-min intervals following each provocation dose until bronchoconstriction resolved.

In addition, blood samples were collected 30 min after administration of ICI 204,219 and at the conclusion of antigen-challenge testing (about 2 h after drug administration). Plasma levels of ICI 204,219 were detected by a high-performance liquid chromatography method with a fluorescence detector that had detection limit of 0.75 ng/ml (Drug Disposition and Metabolism Department, ZENECA Pharmaceuticals Group, ZENECA Inc, Wilmington, Del).

Safety Assessment

Safety was determined from physical examination findings, measurements of vital signs, determinations of subjective symptoms, results of routine clinical laboratory tests, electrocardiographic findings, and spirometry.

Statistical Analysis

Power calculations included information from ten subjects with reproducible baseline data from a previous allergen-challenge study. A sample size of 24 subjects in a three-period crossover design and the reproducibility criterion for baseline eligibility allowed detection of shifts greater than two allergen dilutions in the mean response to the ICI 204,219 formulations at an α level of 0.10 with a power of 80 percent. These levels were chosen to allow a reasonable estimate of equivalence for the protective effects of the aerosol formulations, given the limited number of subjects expected to meet entrance criteria.

Analysis of variance in a three-period crossover design was used to analyze safety data (vital signs measurements, pulmonary function test results, and laboratory test results). Changes in laboratory data within treatments were assessed by paired t tests.

Efficacy data were analyzed as showing dichotomous responses, with challenges classified as censored or not censored. A censored challenge was defined as one in which the maximum allergen-challenge concentration was given without producing a 20 percent decrease in FEV,. This dichotomous classification was used because 31 of 39 challenges were censored, and the analysis of variance technique was inappropriate. Therefore, the baseline was defined as the mean PC20 of the two screening challenges, and the response was assessed as the lowest PC20 during the randomized periods. If the responses to challenges in all three randomized periods were censored, then the maximum concentration was used as the PC20. For each patient, a paired t test of the difference in the log2 transformed PC20 was performed. The effects of the three ICI 204,219 formulations were compared using McNemar’s test.

Table 1—Allergen Concentrations During Bronchoprovocation Testing

<table>
<thead>
<tr>
<th>Provocation No.</th>
<th>Dilution No.</th>
<th>Ragweed Concentration, μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>0.39</td>
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<tr>
<td>5</td>
<td>8</td>
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<td>5</td>
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<tr>
<td>12</td>
<td>1</td>
<td>100.0</td>
</tr>
</tbody>
</table>

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RESULTS

Patient Population

Twenty-two subjects with mild asthma were screened for the study, and 20 subjects had reproducible responses to allergen screening challenges. Sixteen subjects (12 men and 4 women, mean age 31.7 ± 1.8 years) entered the treatment period of the study. Thirteen subjects received doses in all three study periods, one subject received doses in two study periods, and two subjects received doses in only one study period. Overall, 15 subjects received formulation 1, 15 subjects received formulation 2, and 13 subjects received formulation 3. The 13 subjects who received doses in all three study periods were included in efficacy analyses. The three subjects who did not receive each scheduled dose of ICI 204,219 were excluded from the efficacy analysis but were included in the safety analysis.

Fifteen subjects were given medications for allergic rhinitis and asthma during the intervals between allergen challenges, and five subjects received medi-

![Graph](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21689/)
Table 2—Bronchial Challenge Test Results

<table>
<thead>
<tr>
<th>Response Criteria</th>
<th>Form 1 (n = 13)</th>
<th>Form 2 (n = 13)</th>
<th>Form 3 (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No 20% decrease in FEV₁,¹ *</td>
<td>11 (85)</td>
<td>9 (69)</td>
<td>11 (85)</td>
</tr>
<tr>
<td>100 μg/ml allergen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20% decrease in FEV₁,¹</td>
<td>1 (8)</td>
<td>2 (15)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>100 μg/ml allergen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 100 μg/ml allergen</td>
<td>1 (8)</td>
<td>2 (15)</td>
<td>2 (15)</td>
</tr>
<tr>
<td>*Censored response.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Efficacy

All 13 subjects had at least a 20 percent decrease in FEV₁ at both screening challenges, with responses provoked at allergen concentrations ranging from 0.05 to 6.25 μg/ml.

Figure 1 shows allergen-induced changes in FEV₁ for subjects after both screening challenges and challenges after administration of ICI 204,219. Table 2 summarizes bronchial challenge test results by formulation; Table 3 presents bronchial challenge test results for each of the 13 subjects.

The majority of subjects tolerated bronchoprovocation testing without a 20 percent decrease in FEV₁ (31 of 39 challenges). The mean (SE) log shift was 1.6 (0.23), with a 95 percent confidence interval of a 1.0 to 2.2 log shift (10- to 158-fold shift). This was a significant (p = 0.0001) shift from the screening values. No statistically significant differences were observed between formulations. Six subjects had a 20 percent decrease in FEV₁ at end point allergen concentrations greater than fourfold of the screen concentrations. Only two subjects had a 20 percent decrease in FEV₁ at end point allergen concentrations within two dilutions of their maximum screen concentrations; one of the subjects received formulation 2 (four puffs), and the other subject received formulation 3 (one puff).

Responses in seven subjects were censored (inhalation of five puffs of 100 μg/ml without a PC20) at every visit, and all other subjects had censored responses in at least one (n = 1) or two (n = 5) other periods. Thirty-one of 39 individual challenges were censored, which contributed to early termination of the study.

No statistically significant treatment or sequence differences were noted for pulmonary function test results (FEV₁, FVC, or FEV₁ percent predicted) before or after bronchoprovocation.

Plasma concentrations of ICI 204,219 indicated differences in systemic exposure. All subjects who received formulation 1 (n = 15) had detectable drug plasma concentrations 30 min and 2 h after dosing. None of the subjects who received formulation 2 (n = 15) had detectable concentrations after 30 min, and 8 had detectable concentrations after 2 h. Of the subjects receiving formulation 3 (n = 15), 2 had detectable concentrations after 30 min, and 7 had detectable concentrations after 2 h.

Safety

Twenty-seven adverse events were recorded for ten subjects. All adverse events were mild (n = 16) or moderate (n = 11); the most common events were headache (n = 6), rhinitis (n = 4), and cough (n = 3). No serious or unexpected adverse events were attributed to ICI 204,219.

Clinical laboratory tests showed a significant (p = 0.02) treatment difference in white blood cell count, with subjects taking formulation 1 having a significantly lower count than subjects taking formulation 2 (p = 0.006, 5.71 vs 6.35 x 10⁹/L). This difference was not deemed to be clinically significant. Findings from physical examinations, electrocardiograms, and vital signs measurements were not statistically significantly different from baseline after treatment with ICI 204,219.

Discussion

Our results indicate that inhalation formulations of ICI 204,219 successfully inhibited bronchoconstriction in subjects with reproducible sensitivity to ragweed challenges. Inhaled ICI 204,219, given 30 min before ragweed challenge, produced total inhibition of ragweed-induced bronchoconstriction in 31 of 39 challenges in 13 subjects with mild asthma who were tested in the absence of environmental conditions for other indications. None of the subjects received steroids or cromolyn in any form.
exposure to ragweed. There were no differences in the results of antigen bronchoprovocation when patients were tested either 3 days or 4 weeks apart. Antigen-induced bronchoprovocation, therefore, appears to be a useful model to evaluate the efficacy of leukotriene receptor antagonists for the treatment of asthma, provided appropriate steps are taken to ensure the accuracy of the bronchoprovocation test results. In this study, in contrast to a previous study, we required subjects to have two reproducible screening provocations with ragweed before they underwent bronchoprovocation.

ICI 204,219 did not act as a bronchodilator. In preclinical studies, ICI 204,219 was shown to be a potent antagonist of LTD4 receptors, but it had no effect on other receptors. ICI 204,219, administered as a single 40-mg oral dose, was shown to be a potent antagonist of LTD4-induced bronchoconstriction in normal subjects. In our study, inhibition of ragweed-induced bronchoconstriction after administration of ICI 204,219 occurred in the absence of any improvement in FEV1. In addition, changes in plasma concentrations of ICI 204,219 had no effect on blocking ragweed-induced bronchoprovocation, as varying drug plasma concentrations were observed at 30 min and 2 h after dosing for each formulation of drug.

This study was performed in an open-label fashion to compare the three formulations. Either two separate studies or a six-way crossover design would have been necessary to maintain a double-blind study during a limited time period (zero ambient ragweed counts). We had concerns that patients would spontaneously cough or show bronchoconstriction, so we cannot explain our results other than as a valid reflection of protection from allergen-induced bronchoconstriction. Neither patient suggestion nor clinician bias should allow patients to inhale 10 to 158 times more ragweed.

ICI 204,219 is one of several aerosol formulations of leukotriene receptor antagonists to be investigated in humans. FPL 55712, the first available inhaled leukotriene receptor antagonist, produced mild bronchodilation in two of four subjects with asthma, but further application of the drug was limited by its associated throat discomfort. Aerosol formulations of SKF 104,353, a potent antagonist of LTD4 and LTE4, and L-648,051, a selective competitor of LTC4 and of LTD4, are currently being evaluated in clinical studies. Bel et al evaluated the effects of inhaled L-648,051 on the airway response to inhaled antigen in 10 subjects with asthma who inhaled doses of 12 mg of L-648,051 before and 3 h after the allergen challenge. In contrast to the results with ICI 204,219, there was no consistent effect on antigen-induced airway responsiveness. The relative lack of effect with L-648,051 suggested that either the potency or duration of activity of L-648,051 is limited or that LTC4 and LTD4 do not play an important role in human allergic asthma. A subsequent study was conducted with 500 µg of inhaled L-648,051 to determine its ability to prevent and reverse antigen-induced asthma. L-648,051, when given prophylactically, had a slight but significant effect on airway resistance during the early phase but not during the late phase. Some improvement in FEV1 and FVC was observed during the early phase; however, the changes were not statistically significant. When given after the antigen challenge, L-648,051 was not effective in reversing antigen-induced asthma. From these results, the investigators concluded that LTD4 does play a role in the immediate phase of antigen-induced asthma, but that higher doses of L-648,051 should be tested in humans.

ICI 204,219 is the only leukotriene receptor antagonist that has been shown to be effective in blocking bronchoprovocation when given both by the oral and inhaled routes. An oral dose of 40 mg was given 2 h before the cat-dander challenge, whereas only 0.2 mg of inhaled ICI 204,219 was required 30 min before ragweed challenge. In the former study, which used a comparable challenge protocol, oral ICI 204,219 shifted the PC20 significantly, approximately tenfold on average. Two of 13 subjects (15 percent) had censored responses. In contrast, the lower doses of inhaled ICI 204,219 used in this study shifted the PC20 significantly, approximately 46-fold on average. Thirty-one of 39 subjects (79 percent) showed a censored response. These data suggest that the inhaled route of administration may have provided higher local concentrations of active drug in the airway than the oral formulation. Generally, inhaled formulations for existing respiratory therapies have achieved broad acceptance because of their rapid onset of action and improved therapeutic-to-toxic ratio. In addition, inhaled premedications can be given in a more timely or spontaneous fashion than oral medications when a known bronchoconstrictive exposure is occurring.

In conclusion, we have demonstrated that inhalation formulations of ICI 204,219 successfully inhibited bronchoconstriction in subjects with reproducible sensitivity to ragweed challenges.

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