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Ipratropium Bromide Treatment of Experimental Rhinovirus Infection

MICHAEL J. GAFFEY,[1] FREDERICK G. HAYDEN,[1,2] JAMES C. BOYD,[1] AND JACK M. GWALTNEY, JR.[2]*

Departments of Pathology[1] and Internal Medicine,[2] University of Virginia School of Medicine, Charlottesville, Virginia 22908

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The importance of parasympathetic-cholinergic mechanisms in the production of common cold symptoms is not clear. The quaternary ammonium anticholinergic antagonist ipratropium bromide was intranasally administered under double-blind, randomized, placebo-controlled conditions to assess its tolerance and efficacy in reducing nasal hypersecretion in adult volunteers with experimental rhinovirus colds. Ipratropium was sprayed intranasally three times daily (80 μg per treatment) for 5 days beginning 24 h after intranasal inoculation of rhinovirus type 39. Clinical colds occurred in 50% of 30 infected ipratropium recipients and in 76% of 33 infected placebo recipients ($P = 0.04$). The nasal mucus weights tended to be lower for ipratropium-treated persons (mean ± standard deviation, 14.7 ± 15.1 g/5 days) than for placebo-treated recipients (24.7 ± 28.0 g/5 days; $P = 0.076$). Whereas total nasal symptom scores were similar between the two groups, the rhinorrhea score analyzed for each day of treatment showed nonsignificant trends favoring the ipratropium group over the last 4 days of treatment. Ipratropium was generally well tolerated. The results suggest that cholinergic mechanisms are at least partially responsible for nasal mucus production in rhinovirus colds but that the effect of anticholinergic compounds alone is insufficient to be of practical use in treatment, although they may have value as components of multi-ingredient preparations.

Despite recent advances in the molecular characterization of rhinovirus, the pathogenesis of rhinovirus colds remains largely unknown. Since histologic studies have found that rhinovirus causes little direct damage to the nasal mucosa, it has been proposed that the pathogenesis of rhinovirus colds relates to the host response, in particular, activation of the parasympathetic nervous system and release of inflammatory mediators (1, 2, 6, 7, 10, 13, 14, 20, 23, 24). Intranasal administration of ipratropium, a quaternary ammonium cholinergic antagonist, has been shown to be effective in reducing the hypersecretion induced by methacholine challenge (3) and in diminishing the rhinorrhea associated with perennial rhinitis (4). In one small study of persons with colds of undefined etiology, ipratropium reduced paper tissue usage (5). A previous study from this laboratory found that intranasal administration of another quaternary ammonium cholinergic antagonist, atropine methonitrate, was associated with reductions in nasal mucus production during experimentally produced rhinovirus colds (11). The current study was done to further examine whether parasympathetic mechanisms are important in rhinovirus cold pathogenesis. The effects of intranasal ipratropium on nasal mucus production and cold symptoms and its short-term tolerance were examined in otherwise healthy adults with experimental rhinovirus colds.

MATERIALS AND METHODS

Volunteers. Healthy young adult male volunteers with titers of neutralizing antibody to rhinovirus type 39 in serum of ≤1:2 were recruited from the student body of the University of Virginia, Charlottesville. Upper respiratory tract infection or fever of unknown origin within 1 week of the study; concurrent use of oral or intranasal medication; and histories of atopy, sinusitis, asthma, chronic rhinitis, and chronic medical illness were criteria for exclusion.

* Corresponding author.

Viral challenge. In all three trials, rhinovirus type 39 was administered by intranasal drops (0.25 ml per nostril) on two occasions, 4 to 5 h apart. The first virus challenge was given 24 h prior to initiation of treatment. The total virus inoculum was approximately 100 50% tissue culture infectious doses per person.

Drug administration. Ipratropium was formulated in a buffered saline solution to a final concentration of 0.03% (Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Conn.). The vehicle solution served as a placebo. Both formulations were supplied in consecutively numbered, identical-appearing metered-spray devices calibrated to deliver 20 μg of ipratropium per spray. Ipratropium or the placebo was self-administered under observation as two sprays per nostril three times daily for 5 days beginning 24 h after virus challenge. The total ipratropium doses were 80 μg per treatment and 240 μg/day.

Evaluation of illness and infection. The frequency and severity of clinical illness were determined by previously described methods (16, 17, 19) for monitoring clinical symptoms (days 1 to 5 following virus challenge), weighing expelled nasal secretions (days 1 to 5), and counting the numbers of paper tissues used (days 1 to 5). Symptoms were recorded three times per day (morning, afternoon, and evening), and the daily average score was computed for each symptom. In the initial two studies, rhinoscopic examinations for signs of local intolerance were performed prior to treatment and on days 3 and 5 of treatment.

Rates of infection in all three studies were determined by virus isolation and measurement of homotypic neutralization antibody titers from paired specimens, the first taken on the day of virus challenge and the second taken 3 weeks later. Nasal wash specimens were collected prior to virus challenge, on day 1 following challenge prior to initiation of treatment, and on the subsequent 5 days. Nasal wash specimens were collected each morning and used for virus isolation by previously described techniques (16–19). After a

TABLE 1. Tolerance of intranasal ipratropium bromide by rhinovirus-challenged volunteers

Agent (no. of volunteers)	No. of volunteers (% of observation days) with:						
	Dry nose	Dry mouth	Dry eyes	Nasal burning	Other	Dry mucosa[a]	Bleeding sites[a]
Ipratropium bromide (34)	3 (5)[b]	2 (2)	1 (1)	0 (0)	4 (9)[c]	1 (2)	0 (0)
Placebo (35)	0 (0)	0 (0)	0 (0)	1 (3)	1 (1)[d]	0 (0)	1 (3)

[a] Nasal examinations were performed after 2 and 5 days of treatment.
[b] Not statistically significant.
[c] One individual had mild vertigo on day 4; one volunteer had a mild headache on days 2 and 3; two volunteers had mild epistaxis lasting less than 4 min and requiring no treatment on 1 and 2 days each.
[d] One individual had mild epistaxis on 1 day; this lasted less than 5 min and required no treatment.

1-h adsorption period, monolayers were washed twice with 2-ml volumes of Hanks balanced salt solution. All initially negative specimens were recultured on MRC-5 or WI-38 strain human embryonic lung fibroblast cells after one freeze (−70°C)-thaw cycle.

Experimental design. The trial was conducted as a double-blind, placebo-controlled, prospective study. Volunteers were randomly assigned to either treatment or placebo groups by a table of random numbers and isolated in hotel rooms (two volunteers per room) for the study duration beginning 24 h after virus inoculation. Staff members responsible for recording clinical symptoms, collecting clinical samples, and weighing mucus were blind as to the treatment status of the volunteers.

It was not possible to study the projected number of subjects at the same time because of logistical constraints, which included the number of susceptible volunteers available at any one time and the work load of the nursing and laboratory personnel. For this reason, the experiment was performed three times to provide the desired sample size. The sample size was calculated from nasal mucus weight data from an earlier study testing a similar drug (11) to provide statistical power of 80% for detecting a reduction of 40% in mucus weights at an alpha value of 0.05.

Analysis of data. The difference in proportions was calculated by Fisher's exact test, and the difference in symptom scores was calculated by the Mann-Whitney U test. Differences in other measures were calculated by Student's t test by using P values for two-tailed testing.

RESULTS

Tolerance. Although side effects tended to occur more often in drug-treated individuals, intranasal ipratropium was generally well tolerated (Table 1). Nine percent of ipratropium-treated volunteers complained of dry nose on 5% of treatment days, a symptom not reported by placebo recipients. Similarly, the untoward symptoms of dry mouth and eyes were reported by 6 and 3% of ipratropium recipients, respectively, but not by individuals treated with the placebo. Transient nasal burning occurred on 3% of placebo treatment days, compared with 0% of ipratropium treatment days. Mild episodes of epistaxis occurred in two ipratropium recipients and a single placebo recipient and resolved despite continued spray use. Nasal examinations performed during drug administration in the first two efficacy trials failed to show significant differences between the groups.

Infection and illness. As seen previously in the challenge model (13, 14, 19), most volunteers in both the ipratropium and control groups developed infections (Table 2). No antivirus effect of ipratropium spray was noted. Ipratropium-treated volunteers shed virus on 73% of postchallenge observation days compared with 79% of days for those in the placebo group.

Analysis of clinical illness was done only on volunteers who were shown to be infected. Clinical colds, defined by a modification (16) of the criteria established by Jackson et al. (19), occurred in 15 (50%) of 30 ipratropium-treated volunteers, compared with 25 (76%) of 33 placebo recipients (P = 0.04). The mean nasal mucus weights and tissue counts tended to be lower in ipratropium-treated volunteers than in placebo recipients in all three efficacy trials. Nasal symptom scores were similar in the ipratropium and placebo groups. Total nasal mucus weights for the 5 days of collection averaged 14.7 and 24.7, respectively (P = 0.076). Nasal mucus weights analyzed for each day of treatment revealed that ipratropium and placebo recipients produced similar amounts of nasal mucus on treatment days 1 and 2, whereas the ipratropium group tended to produce less mucus than did the placebo group on days 3 and 4 of treatment (Fig. 1A). The rhinorrhea scores analyzed daily showed nonsignificant trends favoring the ipratropium group during the last 4 days of treatment (Fig. 1B).

DISCUSSION

In the current study, volunteers treated with intranasal ipratropium produced 40% less nasal mucus and used 31% fewer tissues than those who received a placebo during the first 5 days after virus inoculation. Trends favoring ipratropium were seen in all three experiments, although the severity of illness was less than expected for the control group in experiment 3. In addition to differences in nasal mucus weights, there were significantly fewer ipratropium recipients who perceived themselves as clinically ill. These findings are similar to those seen with atropine methonitrate, another quaternary ammonium cholinergic antagonist tested by the rhinovirus challenge model (11). We believe that these data provide evidence for a role of the parasympathetic nervous system in the pathogenesis of rhinovirus colds.

Ipratropium bromide, also known under its reference code SCH 1000, is a quaternary isopropyl derivative of atropine with similar pharmacologic properties (8, 9). Selectivity for muscarinic receptors has been reported by Martos et al., who demonstrated similar affinity coefficients for ipratropium, n-methylatropine, and n-methylscopolamine in radioligand-binding experiments on salivary and lacrimal glands (21). In vitro experiments with guinea pig trachea, ileum, and bladder preparations showed that ipratropium possesses little or no antihistaminic properties (J. Offermeier, Abstract, Postgrad. Med. J. **51**[S7]:103–105, 1975). Limited clinical trials have similarly reported that ipratropium is effective in eliminating acetylcholine-induced bronchospasm while offering minimal protection against bronchospasm induced by serotonin or histamine in patients with chronic obstructive pulmonary disease (H. DeVries, Abstract, Postgrad. Med. J. **51**[S7]:106, 1975) and atopy (26; D. Nolte, Abstract, Postgrad. Med. J. **51**[S7]:103, 1975). Thus, the

TABLE 2. Efficacy of intranasal ipratropium bromide or placebo in rhinovirus-infected volunteers

Study and treatment	No. of persons infected/total (%)[a]	No. of colds/no. of persons infected (%)[a]	Days of virus shedding (% of days)[b]	Mean ± SD nasal symptom score (days 1–5)[c]	Mean ± SD nasal mucus wt (g/5 days)[d]	Mean ± SD paper tissue count (no./5 days)[d]
1						
Ipratropium bromide	11/13 (85)	6/11 (55)	53/66 (80)	5.6 ± 3.7	18.6 ± 13.4	47 ± 35
Placebo	15/15 (100)	11/15 (73)	67/90 (74)	5.2 ± 3.7	27.1 ± 30.9	49 ± 39
2						
Ipratropium bromide	10/12 (83)	3/10 (30)	43/60 (72)	3.9 ± 3.8	13.8 ± 15.5	26 ± 29
Placebo	10/10 (100)	7/10 (70)	53/60 (88)	5.3 ± 5.2	29.2 ± 32.5	56 ± 51
3						
Ipratropium bromide	9/9 (100)	6/9 (67)	36/54 (67)	6.0 ± 4.5	11.18 ± 17.04	26.3 ± 47.4
Placebo	8/10 (80)	7/8 (88)	36/48 (75)	7.3 ± 4.6	14.42 ± 12.31	48.1 ± 40.2
Combined						
Ipratropium bromide	30/34 (88)	15/30 (50)[e]	132/180 (73)	5.3 ± 3.9	14.7 ± 15.1[f]	33.6 ± 37.4
Placebo	33/35 (94)	25/33 (76)	156/198 (79)	5.8 ± 4.3	24.7 ± 28.0	48.1 ± 40.2

[a] Infection was determined by virus shedding or seroconversion or both. Colds were defined as a total symptom score of ≥5 during days 1 to 5 after virus challenge and either the presence of rhinorrhea on ≥3 days or the subjective impression of a volunteer that he or she had a cold (16, 19).
[b] Expressed as the number of days positive for virus isolation/total number of days of observation on days 1 to 6 after virus challenge for infected subjects only.
[c] Represents the individual rhinorrhea and nasal congestion and sneezing score totals for days 1 to 5 after virus challenge for all infected subjects.
[d] Mucus weights and tissue counts on days 1 to 5 after virus challenge for all infected subjects.
[e] $P = 0.04$ between the ipratropium bromide and placebo groups by Fisher's exact test; P values for two-tailed testing.
[f] $P = 0.076$ between the ipratropium bromide and placebo groups by Student's t test; P values for two-tailed testing.

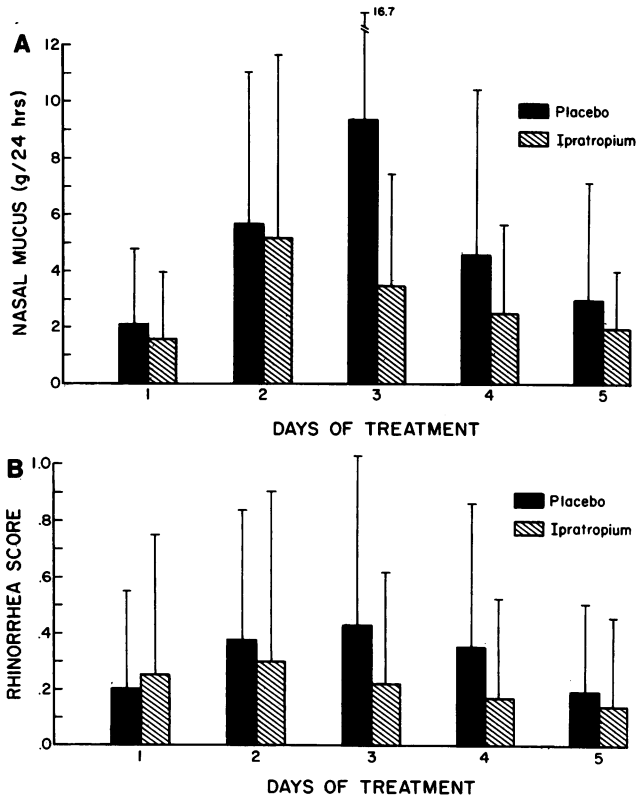

FIG. 1. Mean (± standard deviation) nasal mucus weights (A) and rhinorrhea scores (B) of volunteers with experimental rhinovirus infection treated intranasally with ipratropium or a placebo spray for 5 days. The ipratropium dose used was 80 μg three times a day. Treatments were initiated 24 h after the initial virus challenge.

available evidence indicates that the clinical effects of ipratropium are predominately associated with the competitive antagonism of muscarinic receptors.

It is not known which mechanisms contribute to the total volume of nasal secretions produced in rhinovirus colds. Secretions are composed of material produced both locally by nasal glands under parasympathetic control and by transudation of plasma from nasal vessels. Even if ipratropium were 100% effective in prevention of nasal gland hypersecretion secondary to parasympathetic activation, it would not necessarily be expected to eliminate nasal hypersecretion associated with vascular transudation. Thus, parasympatholytic blockade would not be expected to totally eliminate the nasal discharge associated with rhinovirus colds.

In the current study, the dose of ipratropium (80 μg three times a day) was generally well tolerated, with only a mildly elevated rate of adverse nasal symptoms compared with that seen for the placebo. Rhinoscopic findings were similar between the two groups. It is notable that in the current study, as well as those by Groth et al. (15) and Borum et al. (5), systemic side effects were virtually absent. The relative lack of adverse symptoms was not unexpected, since ipratropium administered either orally or by inhalation is poorly absorbed and since doses of ipratropium many times those required for maximum therapeutic effect fail to affect eye and urinary bladder functions or heart rate (22, 25; A. Bleichert, Abstract, Postgrad. Med. J. 51[S7]:92–93, 1975; G. Scheufler, Abstract, Postgrad. Med. J. 51[S7]:132, 1975; H. W. Thumm, Abstract, Postgrad. Med. J. 51[S7]:32–33, 1975).

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