Long-term effect of ipratropium bromide and fenoterol on the bronchial hyperresponsiveness to histamine in children with asthma

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We studied the effects of the anticholinergic ipratropium bromide (40 μg three times daily) and the β-agonist, fenoterol (0.2 mg three times daily), both administered by powder inhaler, on bronchial hyperresponsiveness (BHR) to histamine in children, aged 7 to 15 years with mild stable asthma and limited bronchoconstriction who had a highly increased BHR. The double-blind, randomized, parallel study was conducted and performed in spring and early summer. BHR and FEV, were measured on two occasions, before the start of treatment and monthly thereafter for 4 months. Symptoms, peak expiratory flow, and concomitant medication were registered daily. Nine of the 12 patients receiving ipratropium bromide and all eight patients receiving fenoterol completed the study. Patients completing treatment had few symptoms and were in a stable condition throughout the treatment period. Neither the administration of ipratropium bromide nor fenoterol resulted in a significant change of BHR. We concluded that long-term treatment with ipratropium bromide or fenoterol had no effect on BHR in children with mild stable asthma. (J ALLERGY CLIN IMMUNOL 1989;84:874-9.)

In asthma and chronic airflow obstruction, airways are hyperresponsive to a variety of pharmacologic and nonpharmacologic stimuli. In adults, BHR is associated with the development of chronic obstructive lung disease and the progressive loss of pulmonary function.[1] BHR may also be a determinant in the outcome of childhood asthma.[2]

The basic mechanisms responsible for BHR are only partially known. In patients with permanent BHR, chronic inflammation of the bronchial wall often appears to be present,[3] but it has not been established whether chronic inflammation and BHR are causally related. A number of studies has demonstrated that BHR was reduced after long-term treatment with inhaled corticosteroids that suppress airway inflammation.[4]

Abbreviations used
BHR: Bronchial hyperresponsiveness
PD_{20}: Dose of histamine that causes a 20% fall in FEV_1
PEF: Peak expiratory flow
t.i.d.: Three times daily

The autonomic nervous system may also be involved in the development of BHR. Results of studies on the administration of a single dose of the anticholinergic, ipratropium bromide, 30 to 90 minutes before challenge with histamine or allergen, are, however, controversial.[5] The only study published so far on the long-term effect of anticholinergic treatment on BHR in children with asthma demonstrated a slight but significant increase in the PD_{20} after treatment with ipratropium bromide, 40 μg t.i.d. for 4 weeks.[6] No reduction in the variability of morning and evening PEF, which is another measure of BHR, was observed.

The purpose of the present study was to assess the effect of long-term treatment with ipratropium bromide on BHR to histamine in children with asthma. We chose fenoterol rather than placebo for the reference group because fenoterol has no effect on BHR[4]

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TABLE I. Patients' characteristics

	Sex	Age (yr)	FVC (% pred)	FEV_1 (% pred)	FEV_1/FVC (%)	Increase in FEV_1 after 0.4 μg of fenoterol (% baseline)
Ipratropium bromide						
1	M	12	103	90	70	18
2	M	9	98	99	79	1
3	M	12	96	93	76	6
4	F	15	114	116	88	7
5	M	10	100	84	70	20
6	M	13	100	93	72	0
7	M	15	93	104	89	16
8	M	14	99	90	72	6
9	M	10	89	92	82	1
10	F	15	119	108	79	15
11	F	9	101	82	70	17
12	M	12	95	96	80	−13
Fenoterol						
1	M	11	98	89	72	13
2	F	13	110	99	78	4
3	M	13	101	91	75	13
4	F	7	98	97	83	4
5	M	11	88	88	79	−2
6	F	12	115	98	75	−2
7	M	15	104	107	82	10
8	M	15	101	97	76	12

% Pred. Percent predicted.
For PD_{20} values, see Table II.

and generally controls asthma effectively in the kind of patients selected for this study.

METHODS

Patients

Twenty patients with atopic asthma, aged 7 to 15 years, selected from the outpatient department of Pulmonary Medicine, Sophia Children's Hospital, University of Rotterdam, were studied. Since BHR in asthma is, to a limited extent, dependent on airway caliber,[7] we selected children with allergic asthma and long-term BHR with minor degree bronchoconstriction. Inclusion criteria were (1) a PD_{20} of ≤150 μg, that is, < -1.65 standard scores from reference PD_{20},[8] (2) FVC and $FEV_1 \geq 80\%$ of predicted, (3) $FEV_1/FVC \geq 70\%$ of predicted, and (4) ≤20% increase in baseline FEV_1 after 0.4 mg of inhaled fenoterol.

The patients had to be capable of performing pulmonary function tests in a reproducible way (i.e., coefficient of variation of FEV_1 in three consecutive measurements ≤5%). All criteria were fulfilled on two occasions in the 4-week period before the start of the study and at least on one earlier occasion.

All patients had mild chronic symptomatic asthma and used bronchodilators or cromoglycate continuously or intermittently. Two patients were taking inhaled steroids. Medication was stopped at least 1 week before the study. Characteristics of the patients are presented in Table I.

The study was performed from February to July 1988. Pollen counts were low in spring and early summer because of the wet climate.

The study protocol was approved by the ethical committee of Sophia Children's Hospital, and informed consent was obtained from each subject and his or her parents.

Pulmonary function and bronchial hyperresponsiveness

FVC and FEV_1 were measured in triplicate with a water-filled 8 L spirometer (Lode D51). The highest of three values was used for data analysis. BHR was measured by inhalation of histamine aerosol in increasing dosages according to a standard protocol used in our laboratory. Tests were performed in each patient at about the same time of the day between 9 AM and 5 PM. Histamine was nebulized with a DeVilbiss 617 nebulizer (DeVilbiss Co., Somerset, Pa.) and a Rosenthal-French dosimeter (The Johns Hopkins University, Baltimore, Md.). Inhaled doses were doubled from 5 μg up to 640 μg as a maximum dose. The effect of each dose was determined by measuring FEV_1 30 seconds after each administration. PD_{20} was calculated by linear interpolation of data points from a log dose-response curve. With this technique we found in our laboratory a mean ± SD of

TABLE II. PD_{20} (micrograms) before and during treatment

	Treatment					
	Before		During (mo)			
Patient No.	1	2	1	2	3	4
Ipratropium bromide						
1	13	19	3	11	8	36
4	40	53	42	38	37	
6	60	32	56	80	56	46
7	21	28	40	26	90	50
8	54	40	15	36	7	13
9	115	26	35	85	170	90
10	8	9	7	15	25	140
11*	6	4	7	11	9	12
12	75	100	48	160	90	95
Mean $\log_{10}$ PD_{20}	1.463	1.392	1.282	1.539	1.506	1.649
SD	0.453	0.414	0.462	0.414	0.513	0.391
Geometric mean	44	34	28	51	55	60
Fenoterol						
1	14	28	21	1	10	13
2	38	15	20	13	32	215
3	10	9	24	7	14	24
4	6	16	5	13	5	7
5	11	11	10	9	7	28
6*	40	20	70	110	215	130
7	36	22	11	24	30	14
8	17	10	8	7	7	19
Mean $\log_{10}$ PD_{20}	1.242	1.183	1.187	1.037	1.231	1.457
SD	0.308	0.175	0.354	0.573	0.533	0.511
Geometric mean	22	16	21	23	40	56

*Significant correlation between $\log_{10}$ PD_{20} and duration of treatment.

the $\log_{10}$ PD_{20} (in micrograms) in healthy children without current or past history of respiratory disease of 2.93 ± 0.25, corresponding to a mean PD_{20} of 850 μg. Patients were regarded as having a highly increased BHR if PD_{20} was <150 μg (3 SD below the predicted mean) and having a slightly increased BHR if PD_{20} was between 150 and 270 μg (between 3 and 2 SD below the predicted mean). The 24-hour within-subject reproducibility of this technique was found to be one doubling dose.[9] Measurements were only performed if the baseline FEV_1 was not <90% of the lowest prestudy value to ensure that histamine provocation tests were done at values of baseline airway caliber that were comparable throughout the treatment period. Pulmonary function and BHR were measured twice in the month before the start of the study and at monthly intervals thereafter when the children visited the outpatient clinic. All medication was stopped about 12 hours before each measurement of pulmonary function and BHR.

Treatment

Patients were entered into a double-blind, randomized study of two parallel groups. Twelve patients were allocated to ipratropium bromide (Atrovent; Boehringer Ingelheim, Alkmaar, The Netherlands) at a dose of 40 μg, and eight patients were allocated to fenoterol (Berotec; Boehringer Ingelheim) at a dose of 0.2 mg, both administered t.i.d. by dry-powder inhaler. Allocation and regulation of group size were done by the sponsor of the study who provided each patient with coded boxes containing the drug. A placebo-treated-only group was not considered feasible. Treatment was administrated for 4 consecutive months. The inhalation technique was checked before the study and at each visit to the clinic, and the number of capsules used was also checked. Since it was our primary purpose to test the effect of ipratropium bromide, we wanted to have more patients receiving the anticholinergic than the β-agonist, and we therefore decided to take the maximally allowed inequality of group size.[10]

Salbutamol by metered dose or powder inhaler was the only rescue medication allowed during the study.

Children were required to register cough, wheezing, and asthmatic attacks on a daily record card giving a score of severity between 0 and 3 for each item. Concomitant medication, side effects, and PEF rate before drug in the morning and evening were also noted.

Data analysis

PD_{20} is presented in the text in micrograms, but $\log_{10}$ transformations were applied to the PD_{20} values before statistical evaluation. FEV_1 was expressed as a percentage of the predicted value.[11] Symptoms were expressed as the daily sum of scores for cough, wheezing, and asthmatic attacks.

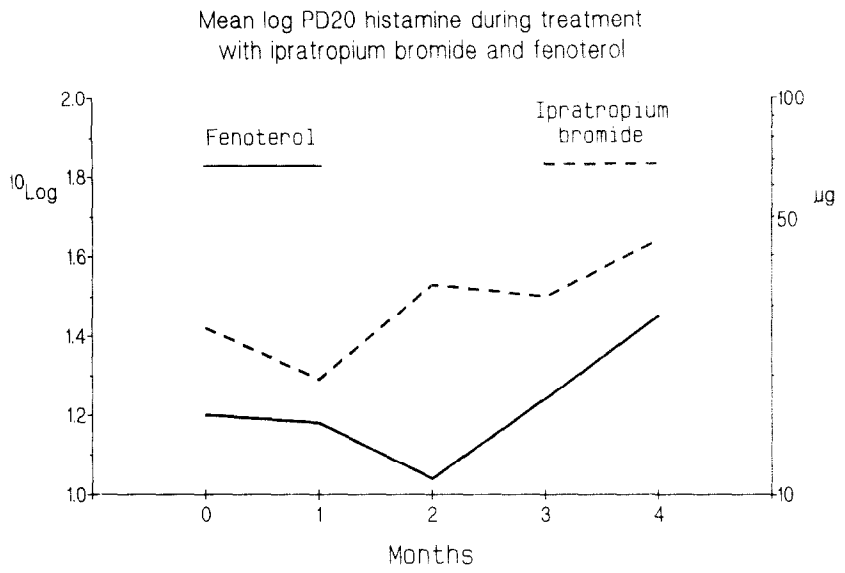

FIG. 1. Mean $\log_{10}$ PD_{20} histamine during treatment with ipratropium bromide and fenoterol.

Student's *t* test for unpaired data and Fisher's exact test were used to compare the two treatment groups at entry in the study.

We tested the null hypothesis that both treatment groups behaved in a similar way, as regards BHR.

An individual change in PD_{20} was represented by the estimated slope of the linear regression between $\log_{10}$ PD_{20} and duration of treatment. To this end, the slope or trend of PD_{20} with time was estimated for each individual. The Mann-Whitney U test was used to analyze whether there was a significant difference between the two treatment groups in the distribution of these slopes ($\alpha = 0.10$; two-sided). Similar analyses were performed for the slope of symptom scores with time, the slope of diurnal PEF variability with time, and for the slope of PD_{20} with FEV_1. Sign tests were used to estimate whether positive or negative slopes predominated significantly in the treatment groups. Changes were considered to be of clinical significance if BHR diminished from highly increased to slightly increased or normal. On pooled data from both treatment groups, Spearman's rank-correlation coefficient was used to test the association between the baseline PD_{20} and the time trend of PD_{20} within individuals. A negative correlation was considered as indicative of "regression-to-the-mean."

RESULTS

The two groups were comparable with respect to age, sex, and baseline lung function (Table I) and PD_{20} (Table II) at entry into the study. The time behavior of mean PD_{20} in the two groups during treatment is illustrated in Fig. 1.

Estimated slopes of PD_{20} values within individuals with time were not significantly different between the two treatment groups ($p = 0.3$). No significant negative rank correlation was found between baseline PD_{20} and its slope with time. In the fenoterol-treated patients, PD_{20} and FEV_1 values demonstrated a significant positive correlation within individuals ($p = 0.003$), but this was not the case for patients taking ipratropium bromide ($p = 0.9$). The effect of treatment with ipratropium bromide and fenoterol on the relationship between values of PD_{20} and FEV_1 was, however, not significantly different ($p = 0.4$).

Mean weekly symptom scores varied between 0 to 2.7/day and were similar in the children receiving ipratropium bromide and children receiving fenoterol. Treatment groups demonstrated no difference in PEF diurnal variability. Mean FEV_1 remained approximately unchanged with ipratropium bromide and increased 6% after fenoterol administration.

In the ipratropium bromide-treated group, three patients were withdrawn. Two of these patients (Nos. 2 and 3) had an increase in asthma symptoms some weeks after entering the study; both patients were previously receiving maintenance therapy with inhaled steroids. Patient No. 5 was receiving continuous therapy with inhaled cromoglycate that was stopped on entering the study; there was a gradual worsening of his clinical condition, and FEV_1 decreased to 60% of predicted after 1 month. No side effects were encountered.

DISCUSSION

The mechanisms underlying BHR are complex.[12-14] BHR manifests itself as a chronically elevated baseline airway sensitivity to bronchoconstricting agents, which in their turn may cause a further transient increase in responsiveness. In asthma, genetic factors appear to be involved in the development of BHR.[15] It appears likely, however, that the expression and persistence of BHR mainly depend on non-

genetic factors, among which is mild airway inflammation.[3]

Studies in adults and children indicate that BHR may be an independent determinant of the outcome of chronic airway disease.[1, 2] The question is therefore whether BHR can be reduced by drug treatment and whether this will result in an improvement of outcome. We attempted to study the effect of an inhaled anticholinergic, that is, the effect of ipratropium bromide on baseline BHR, which is the component that remains when patients are in a stable condition for a long period without any transient increase in symptoms. To this end we selected children with stable, well-controlled asthma in whom bronchial obstruction and bronchoconstriction were mild but who demonstrated a highly increased bronchial responsiveness. In a previous study we have demonstrated that BHR in children with similar characteristics will improve and may even normalize after treatment with inhaled steroids, but not with a β-agonist.[4]

A possible effect of ipratropium on histamine responsiveness could be expected for various reasons. Responsiveness to histamine is indirectly mediated via receptors in the airway mucosa that activate vagal nerve fibers and directly via histamine receptors on smooth muscle.[16] Blockade of muscarinic receptors might therefore result in a diminished responsiveness to histamine. Furthermore, Daniel et al.[17] have suggested that parasympathetic nerves may interact with mast cells close to smooth muscle. An increase in parasympathetic nerve activity might therefore enhance mast cell histamine release, resulting in airway hyperresponsiveness to histamine. This would be reversed by blockade of the parasympathetic receptor on mast cells.

We also considered the possibility that long-term administration of ipratropium might cause up regulation of muscarinic receptors,[18] resulting in an increase rather than a decrease in histamine responsiveness. Results of studies on the acute inhibitory effect of ipratropium on histamine-induced bronchoconstriction are conflicting. Most investigators found no or only a small effect.[5, 19] For β-agonists, it has been demonstrated that bronchodilation is not the major mechanism responsible for the protection against histamine responsiveness.[20] This finding appears also to be true for ipratropium.[19] In this last study, doses ranging from 5 to 1000 μg were found to cause a dose-related increase in FEV_1 but no significant change in PD_{20} to histamine. In view of these findings, we did not consider it as a problem that histamine responsiveness was measured after the acute bronchodilator effect of the drugs administered had worn off, as appeared from the unchanged FEV_1 at the time of the test. The study by Newcomb et al.[18] indicates, however, that the effect of ipratropium on muscarinic receptors may last for up to 72 hours. Administration of 60 μg of ipratropium, four times a day for 3 weeks in young adult subjects with mild asthma, resulted in a PC_{20} to methacholine that was significantly lower at 24 hours after the last dose compared to premedication baseline, and returned to baseline within 48 to 72 hours, indicating receptor upregulation. Twelve hours after withdrawal, sensitivity to methacholine was still reduced. With histamine, we found no indications for an up regulation of muscarinic receptors. Vathenen et al.[21] recently suggested that treatment for 2 weeks with terbutaline impaired the ability of the β-agonist to protect against histamine-induced bronchoconstriction and was followed by a slight rebound increase in BHR after cessation of therapy. They interpreted this as indicative of β-receptor down regulation. Their findings were based on repeated measurements of PD_{20} during 24 hours. We measured PD_{20} at only one point of time, that is, 12 hours after stopping treatment. The increase in BHR found by Vathenen et al.[21] at that point of time was small and not of clinical significance. It is unlikely therefore that an eventual rebound phenomenon will have influenced our results in a way that would interfere with our conclusions.

Although the lack of an acute effect makes a long-term protection less likely, this finding has never been studied except by Sly et al.[6] These investigators noticed an increase in mean PD_{20} to histamine from 0.49 mg/ml to 0.78 mg/ml after 40 μg of ipratropium bromide t.i.d. for 4 weeks in 31 children with asthma, despite an 8-hour gap between the last dose of ipratropium and the histamine challenge test and the absence of a bronchodilator effect. These results could suggest an anticholinergic effect on histamine responsiveness that lasted longer than the bronchodilation. It appears, however, also possible that the difference in PD_{20} values was not due to a specific drug effect but to the variability of the measurement that has a 95% range of a single value of 2.11 doubling doses with the technique used.[22] Furthermore, an eventual effect of concomitant medication (salbutamol, theophylline or inhaled beclomethasone) cannot be excluded, since no details at this point were provided in the article. As perhaps could be expected, the results of Sly et al.[6] were not confirmed in the present study. As in a previous study,[4] we also found β-receptor stimulation to have no effect on BHR.

A reason for our negative findings might be that the dose of ipratropium bromide used was too low. We gave a dose that is double that recommended for treatment in adults and that we often find to be effective in children. We considered this adequate, which is supported by the fact that symptoms of asthma were well controlled in the nine patients re-

ceiving ipratropium bromide and the eight children receiving fenoterol who completed the study. Most patients' baseline airway caliber remained unchanged. Six patients experienced a change in FEV_1 between 10% and 15% of baseline. One patient in the fenoterol-treated group experienced a change of 22% (patient No. 3). PEF variability was not significantly affected by ipratropium bromide or fenoterol. This indicates that symptoms of asthma in the children who completed the study were mild and stable throughout the treatment period and that the conditions under which airway responsiveness was measured were comparable to conditions in the period before the study. It appears therefore likely that we have indeed fulfilled our purpose to assess the effect of ipratropium bromide and fenoterol on baseline BHR.

These data of the patients from the ipratropium bromide-treated group who were withdrawn (Nos. 2, 3, and 5) are not included in Table II and Fig. 1. Patients Nos. 2 and 3 had mild asthma with a PD_{20} of 27 and 40 μg, respectively. Patient No. 5 had moderate but well-controlled asthma with a PD_{20} of 40 μg. Hence, the characteristics of the patients who were withdrawn did not differ from characteristics of the patients who completed the study. It is therefore unlikely that these patients would have influenced the conclusions had they completed treatment.

Data from longitudinal studies can be analyzed in various ways. Because it was difficult to find patients who fulfilled the inclusion criteria and were also willing to participate in a long-term study, the number of children included was rather small. This limits the power of the study. By analyzing individual slopes of PD_{20} with time, all data points collected within one subject were used. Hence, in this way we have selected a method that was sufficiently powerful to confirm or reject the null hypothesis.

In conclusion, no significant changes of BHR to histamine were found during a treatment period with ipratropium bromide of 4 months in children with mild to moderate chronic symptomatic asthma. Also, fenoterol had no effect on BHR. This raises the question whether bronchodilator drugs like β-agonists or anticholinergics should not be considered anymore as drugs of first choice in the maintenance therapy of asthma. Further studies are needed to answer this question.

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