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Effect of Terfenadine and Ipratropium Bromide on Ultrasonically Nebulized Distilled Water-induced Asthma

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Inhalation of ultrasonically nebulized distilled water was used to evaluate non-specific bronchial reactivity and to investigate non-immunologically mediated asthma. Release of histamine and other mediators from mast cells and stimulation of lung irritant receptors are mechanisms that may be involved in nebulized distilled water-induced bronchoconstriction. To investigate the contribution of these mechanisms the effect of terfenadine and ipratropium bromide on the bronchial response to this stimulus was assessed. A total of 30 asthmatics (mean 28.7 years) were submitted on three different days to distilled water challenge with or without prior treatment with oral terfenadine or inhaled aerosolized ipratropium bromide. The decrease in forced expiratory volume in 1 s induced by the same dose of distilled water was significantly ($P < 0.001$) reduced from 35.6 ± 15.8% to 19.5 ± 16% with terfenadine and to 23.9 ± 19.7% with ipratropium bromide. The results suggest that histamine release and reflex bronchoconstriction are mechanisms involved in asthma induced by ultrasonically nebulized distilled water.

A inalação de água destilada em nebulisação ultrassónica é um método para avaliar a hiperreactividade brônquica inespecífica e investigar a asma mediada por mecanismos não imunológicos. A libertação de histamina dos mastócitos e a estimulaçao das terminações irritativas

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pulmonares parecem estar envolvidas na broncoconstrição induzida pel a água destilada em nebulisação ultrassónica. Investigamos a contribuição destes mecanismos, avaliando o efeito da terfenadina e do brometo de ipratrópio na resposta brônquica à água destilada. Submeteram-se 30 asmáticos (idade média de 28,7 anos) a provocação com água destilada em tres dias diferentes, sem premedicação e após medicação com terfenadina oral ou brometo de ipratrópia em aerosol. Verificou-se uma reduçao significativa ($P < 0.001$) da queda do volume expiratório máximo por segundo induzida pela mesma dose de água destilada, após administraç ão de ambos os fármacos 35,6 ± 15,8%, para 19,5 ± 16,0% com terfenadina e para 23,9 ± 19,7% com brometo de ipratrópio. Os resultados sugerem que a libertação de histamina e a broncoconstrição reflexa estão envolvidas na asma induzida pela água destilada em nebulisação ultrassónica.

KEY WORDS: Asthma; histamine; reflex bronchoconstriction; terfenadine; ipratropium bromide.

INTRODUCTION

Inhalation of ultrasonically nebulized distilled water induces bronchoconstriction in many patients with asthma[1 - 3] and is used to evaluate non-specific bronchial reactivity. The mechanisms by which hypotonic solutions induce asthma are unclear but release of mast cell mediators, such as histamine,[4, 5] and stimulation of cholinergic pathways[2, 6, 7] are possibilities. Anticholinergic agents have been used to assess whether or not cholinergic pathways are involved in the response but the results are controversial.[1, 6, 8 - 10] Atropine inhibited nebulized distilled water-induced bronchoconstriction in some, but not all, reactive patients.[6, 8] Adequate doses of the anticholinergic agent ipratropium bromide inhibit reflex bronchoconstriction, with a peak effect after 1 h,[9, 10] but 80 μg ipratropium bromide did not significantly reduce the response to nebulized distilled water challenge.[1]

Terfenadine, a selective histamine H_1-receptor antagonist, which is devoid of central nervous system effects[11 - 13] and anticholinergic activity[14, 15] at doses that substantially inhibit the bronchoconstrictor response to exogenous histamine, is a pharmacological tool that can be used to assess whether or not endogenous histamine is involved in the response to nebulized distilled water. The purpose of the present study was to assess and compare the effects of oral terfenadine and inhaled ipratropium bromide on bronchoconstriction induced by nebulized distilled water in patients with asthma and thereby determine whether histamine or cholinergic reflex pathways contribute to the response.

PATIENTS AND METHODS

Patients

A total of 30 asthmatic patients (13 men, 17 women), aged between 14 and 53 years (mean 28.7 years), participated; all but three were atopic, as assessed by skin prick testing, 16 had mild asthma controlled by inhaled β-adrenoceptor agonists as required and 14 had moderate asthma requiring regular medication with a β_2-adrenoceptor agonist alone or in combination with theo-

phylline or an inhaled corticosteroid. None of the patients smoked. The patients were all in a stable condition and had not suffered from a respiratory tract infection in the 4 weeks prior to the study. Drugs likely to interfere with the study were stopped for the recommended withdrawal times before the challenge.[16]

Induction of asthma

Nebulized distilled water challenge was carried out by sequential inhalation of distilled water at room temperature for three periods each lasting 3 min using a HEYER-USE 77 ultrasonic nebulizer with a fluid output of 3 ml/min. Patients, who wore nose clips, were connected to the nebulizer via a mouthpiece and were instructed to breathe naturally. The distilled water challenge was performed at a similar time on each study day. Day 1 was a control day and no treatment was given; days 2 and 3 were treatment days.

Treatment

Tablets of 60 mg terfenadine (Triludan®) (total dose 180 mg) or ipratropium bromide, administered via a controlled dosage nebulizer dispensing 40 µg puff (total dose 120 µg), were given to the patients 3 or 1 h, respectively, before challenge. On day 2, 180 mg terfenadine or 120 µg ipratropium bromide was administered and the alternative drug was given on day 3, according to a randomization schedule. There was 1 week between study days. Treatments were open, randomized and crossed over. Patients were asked to report adverse events.

Clinical assessment

The forced expiratory volume in 1 s (FEV_1) was measured using a dry spirometer (Vitalograph) before the challenge, immediately after each period of inhalation, and 5, 10 and 15 min after challenge. The test was stopped at the end of the first (3 min) or second period (total 6 min) if a fall in FEV_1 of 20% or more compared with the pre-challenge value was obtained; otherwise, the test was continued for a third 3-min period (total 9 min).

Statistical analysis

An independent analysis was undertaken by the Applied Statistics Research Unit, University of Kent, UK.

Pre- and post-challenge FEV_1 data were analysed, and it was revealed that pre-challenge values for each of the two treatments (day 2 or 3) were higher than control (day 1) values by a mean of approximately 8%, which is consistent with a reduction in bronchomotor tone due to terfenadine and ipratropium bromide. It was inappropriate, however, to apply a statistical test to assess the significance of these differences, instead, 'bronchodilation' (i.e. the difference between pre-challenge FEV_1 on day 2 or 3 and control day) served as a covariate in a comparison of pre-challenge FEV_1 for days 2 and 3. Post-challenge FEV_1 data revealed that the challenge lasted a minimum of 3 min on all occasions. To obtain a balanced data set in which all patients would have comparable data, therefore, only post-challenge FEV_1 measurements collected immediately after distilled water challenge for 3 min were used. All statistical tests were carried out with a two-sided alternative hypothesis and were considered significant at the 0.05 level.

Pre-challenge FEV_1. Analysis of pre-challenge FEV_1 was carried out in two stages. In the first the control day measurements were ignored and an analysis of variance method was applied.[17] This approach uses information from two sources. The between-subject source gives information on the differences between the two groups which in a 2 x 2-crossover design is equivalent to

Triludan® is the registered tradename of Merrel Dow, USA.

carryover effect. The within-subject source contains information on any period or treatment effect.

In the second stage the control day measurements of FEV_1 were utilized according to a method described by Kenward and Jones.[18] The analysis uses control day measurements to improve the test for carryover effect and also gives a direct test for treatment effect. The test for carryover effect involves using the control day measurements as a covariate, whereas the test for direct treatment effect uses the sum of the two treatment measurements as a covariate as well as the control day measurement.

Post-challenge FEV_1. The methods of analysis of post-challenge FEV_1 follow those used for pre-challenge FEV_1 but also included an analysis with bronchodilation as a covariate. Bronchodilation was calculated as the difference between the pre-challenge FEV_1 for the treatment day and the pre-challenge FEV_1 for the control day. Reduction in FEV_1 induced by distilled water challenge was plotted against bronchodilation to assess any relationship visually.

Duration of distilled water challenge. Although statistical analysis of post-challenge FEV_1 data was carried out only on values collected immediately after distilled water challenge for 3 min, on many occasions challenge lasted 6 or 9 min. Descriptive statistics were, therefore, generated for individual patients with respect to the duration of challenge (3, 6 or 9 min) on control and treatment days and for differences in duration (–6, –3, 0, +3 or +6 min) on each treatment day compared with control but statistical tests were not applied.

RESULTS

Pre-challenge FEV_1

Values for FEV_1 for individual patients are given in Table 1. Mean values are illus-

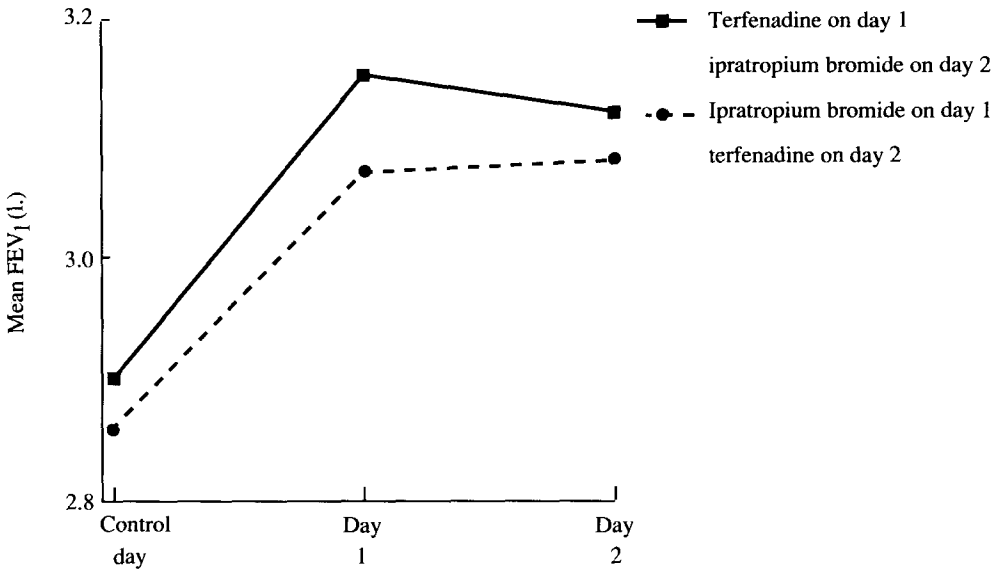

Fig. 1. Effect of 180 mg oral terfenadine and 120 mg inhaled ipratropium bromide on mean pre-challenge forced expiratory volume in 1 s.

Table 1
Effect of 180 mg oral terfenadine and 120 μg inhaled ipratropium bromide on forced expiratory volume in 1 s

	Control day		Period 1		Period 2	
Patient no.	**Pre-challenge**	**Post-challenge**	**Pre-challenge**	**Post-challenge**	**Pre-challenge**	**Post-challenge**
Terfenadine on day 1 and ipratropium bromide on day 2						
1	2.20	0.95	2.35	1.80	2.55	2.10
5	3.60	3.05	3.98	3.68	4.10	2.60
7	3.15	3.05	3.50	3.33	3.50	3.05
8	3.25	2.00	2.40	2.10	3.05	2.23
10	3.20	2.30	3.53	3.20	3.53	1.73
13	4.45	2.23	4.73	4.40	4.60	2.23
15	3.50	3.40	3.50	3.50	3.25	3.05
16	3.80	2.05	4.05	4.00	4.15	3.88
18	1.35	1.30	1.53	1.30	1.40	1.18
19	2.05	1.10	2.55	1.50	1.90	0.98
22	3.05	2.45	3.20	2.85	3.15	3.05
23	2.03	1.30	2.75	1.65	2.65	1.40
25	2.75	2.35	2.75	2.45	3.05	2.00
26	2.80	2.45	3.05	2.80	3.00	3.00
27	2.65	1.20	3.25	2.80	2.70	2.50
Ipratropium bromide on day 1 and terfenadine on day 2						
2	2.60	1.20	3.00	1.85	3.28	2.70
3	2.25	1.48	2.10	1.33	2.50	1.50
4	2.90	2.10	3.35	3.30	3.10	2.98
6	2.85	2.15	2.95	2.65	2.73	2.83
9	2.35	0.80	2.55	2.28	1.65	0.45
11	3.93	3.55	4.44	4.25	4.45	4.13
12	3.68	3.18	3.90	3.43	3.70	3.35
14	2.30	1.80	2.35	2.25	1.95	1.40
17	2.23	1.35	2.40	1.75	2.40	1.90
20	3.45	3.03	3.65	3.40	3.80	3.75
21	2.75	0.85	2.80	0.85	3.13	2.50
24	1.45	0.55	2.13	0.98	2.30	1.15
28	3.45	3.00	3.40	3.45	3.68	3.45
29	3.65	1.78	3.85	1.95	4.20	3.05
30	3.15	2.50	3.25	2.88	3.35	3.08

Table 2
Initial analysis of pre-challenge forced expiratory volume for 1 s using Hills and Armitage analysis[17]

Source	Degrees of freedom	Sum of squares	Mean squares	*F*	*P*
Group	1	0.0315	0.0315	0.03	0.8719
Error	28	33.3154	1.1898		
Period	1	0.0030	0.0030	0.05	0.8176
Treatment	1	0.0065	0.0065	0.12	0.7347
Error	28	1.5556	0.0556		
Total	59	34.9120			

trated in Fig. 1. Results of the Hills and Armitage method of analysis[17] are summarized in Table 2 and results of the Kenward and Jones method of analysis[18] are summarized in Table 3. There was no significant difference between the effect of terfenadine (3 h post-treatment) and of ipratropium bromide (1 h post-treatment) on the pre-challenge FEV_1.

Post-challenge FEV_1

Individual absolute values of FEV_1 are given in Table 1. Mean values for reduction in FEV_1 induced by distilled water are illustrated in Fig. 2. Results of the Hills and Armitage method of analysis[17] are summarized in Table 4. Results of the Kenward and Jones method of analysis[18] are shown in Table 5 with control day data as covariate

Table 3
Analysis of pre-challenge forced expiratory volume for 1 s using control day measurements based on Kenward and Jones analysis[18]

Source	Degrees of freedom	Sum of squares	Mean squares	*F*	*P*
Carryover effect					
Control	1	14.6462	14.6462	105.08	0.0001
Group	1	0.0001	0.0001	0.00	0.9676
Error	27	2.0271	0.0751		
Total	29	16.6735			
Direct treatment effect					
Control	1	0.0065	0.0065	0.92	0.3467
(Per 1 + per 2)	1	0.0050	0.0050	0.72	0.4047
Group	1	0.0010	0.0010	0.14	0.7102
Error	26	0.1828	0.0070		
Total	29	0.1953			

Table 4
Initial analysis of the reduction in forced expiratory volume for 1 s induced by nebulized distilled water using Hills and Armitage analysis[17]

Source	Degrees of freedom	Sum of squares	Mean squares	*F*	*P*
Group	1	0.0005	0.0005	0.00	0.9716
Error	28	11.1044	0.3966		
Period	1	0.3118	0.3118	1.63	0.2120
Treat	1	0.9065	0.9065	4.74	0.0380
Error	28	5.3518	0.1911		
Total	59	17.6750			

and in Table 6 with bronchodilation as covariate. The plots of bronchodilation versus reduction in FEV_1 are illustrated in Fig. 3.

There was a significant treatment effect in favour of terfenadine ($P = 0.046$) for the reduction in FEV_1 induced by distilled water. This effect was confirmed whether or not control day measurements ($P = 0.046$) or bronchodilation ($P = 0.045$) were used as covariates. There were no other effects. The mean reduction in FEV_1 in the presence of terfenadine was 0.46 l (SE ± 0.08), which was significantly different from the mean reduction of 0.70 l (SE ± 0.08) in the presence of ipratropium bromide. Hence, terfenadine inhibited the fall in FEV_1 induced by nebulized distilled water more

Table 5
Analysis of the reduction in forced expiratory volume for 1 s induced by nebulized distilled water using control day measurements

Source	Degrees of freedom	Sum of squares	Mean squares	*F*	*P*
Carryover effect					
Control	1	1.9740	1.9740	14.93	0.0006
Group	1	0.0075	0.0075	0.06	0.8142
Error	27	3.5710	0.1323		
Total	29	5.5525			
Direct treatment effect					
Control	1	0.0002	0.0002	0.01	0.9349
(Per 1 + per 2)	1	0.0209	0.0209	0.83	0.3694
Group	1	0.1104	0.1104	4.41	0.0456
Error	26	0.6509	0.0250		
Total	29	0.7824			

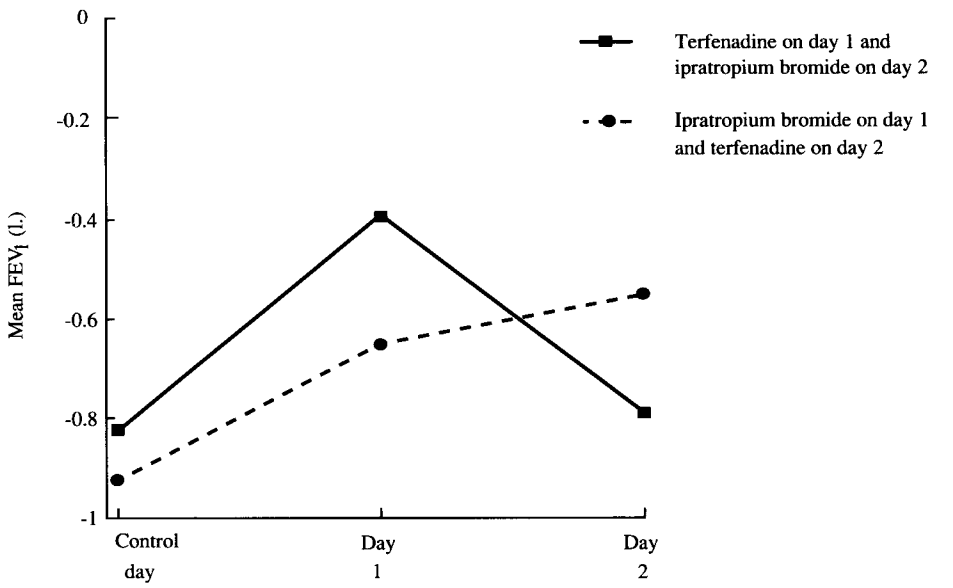

Fig. 2. Effect of 180 mg oral terfenadine and 120 μg inhaled ipratropium bromide on reduction in forced expiratory volume in 1 s induced by distilled water challenge.

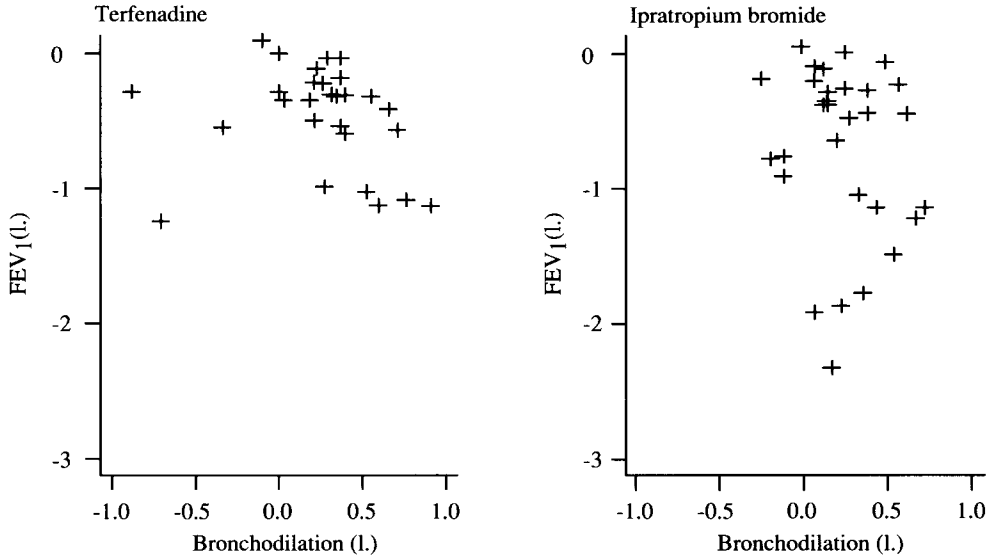

Fig. 3. Effect of 180 mg oral terfenadine and 120 μg inhaled ipratropium bromide on the relationship between reduction in forced expiratory volume in 1 s induced by nebulized distilled water and bronchodilation.

than did ipratropium bromide.

Control day measurements contributed very little to the comparison of treatments (Table 5). Bronchodilation did not contribute significantly to the variation in the reduction in FEV_1 induced by distilled water (Table 6). The plot of bronchodilation versus reduction in FEV_1 suggests that when the bronchodilator effect of terfenadine is large the reduction in FEV_1 induced by distilled water is also large.

Duration of distilled water challenge

Individual values for total duration of distilled water challenge and the difference between duration of challenge post-treatment and on the control day are provided in Table 7. Following terfenadine and ipratropium bromide, 14 and 13 patients respectively, tolerated nebulized distilled water for an additional 3 and 6 min compared to the control day. The numbers of patients who were challenged for an additional 3 or 6 min in the presence of one treatment compared with the other were 10 for terfenadine and three for ipratropium bromide. Furthermore, 17 patients completed distilled water challenge for a total of 9 min in the presence of terfenadine compared with 10 in the presence of ipratropium bromide and only five on the control day.

Safety data

No adverse events were reported.

DISCUSSION

The airways of asthmatic patients, but not of normal subjects, respond to the inhalation of nebulized distilled water.[1-3, 19, 20] The mechanisms by which this stimulus induces asthma is probably provoked partly by a non-immunological release of mediators from mast cells in the airways. It is known that asthmatic patients have mast cells in the lumen[21] and that these cells can be induced to release more histamine than cells from normal subjects.[22] *In vitro* studies have shown that mast cells release histamine when bathed in hypotonic solutions,[4, 23, 24] presumably as a result of movement of water into cells, leading to degranulation. It has been shown that blood levels of histamine and neutrophil chemotactic factor increase in asthmatics after inhalation of nebulized distilled water,[5] analogous to the way in which both mediators are increased after exercise,[25, 26] and this is accompanied by bronchoconstriction. These facts suggest that both stimuli might provoke asthma through non-immunological activation of mast cells in the airways.

The contribution of individual media-

Table 6
Analysis of the reduction in FEV_1 induced by nebulized distilled water using bronchodilation as a covariate

Source	Degrees of freedom	Sum of squares	Mean squares	*F*	*P*
Group	1	0.0005	0.0005	0.00	0.9716
Error	28	11.1044	0.3966		
Dilation	1	0.1655	0.1655	0.85	0.3643
Period	1	0.2956	0.2956	1.52	0.2282
Treat	1	0.8637	0.8637	4.44	0.0445
Error	27	5.2481	0.1944		
Total	59	17.6778			

Table 7
Effect of 180 mg oral terfenadine and 120 μg ipratropium bromide on the duration of nebulized distilled water challenge

Patient no.	Control	Terfenadine	Ipratropium bromide
1	3	3	6 [a]
2	3	6 [a]	3
3	3	3	3
4	3	9	9
5	9	9 [a]	3
6	3	9 [a]	6
7	9	9	9
8	3	9 [a]	3
9	3	3	6 [a]
10	3	9 [a]	3
11	6	9	9
12	6	9	9
13	3	9 [a]	3
14	3	3	9 [a]
15	9	9 [a]	6
16	3	9 [a]	6
17	3	3	3
18	9	6	6
19	3	3	3
20	6	9	9
21	3	3	3
22	3	9	9
23	3	3	3
24	3	3	3
25	6	6 [a]	3
26	6	9	9
27	3	9	9
28	9	9	9
29	3	3	3
30	3	9 [a]	6

[a] Ultrasonically nebulized distilled water challenge was longer in duration in the presence of terfenadine (10 patients) or ipratropium bromide (three patients) compared with the other treatment.

tors to bronchoconstriction induced by several agents can be investigated by using drugs that inhibit synthesis of the mediator or specifically antagonize its effects on target tissues. Although histamine is only one of the mediators involved in the asthmatic response to allergens and various physical agents, large doses of H_1-blockers can reduce the asthmogenic effect of some of the triggering factors. Histamine is, therefore, a mediator with pathophysiological relevance.[27] Studies of the action of antihistamines on the bronchial tree are difficult to perform because of central nervous system effects. The introduction of new antagonists, such as terfenadine, with potent pharmacological activity on H_1-receptors but which are devoid of sedative or anticholinergic side-effects has renewed interest in the use of H_1-receptor antagonists in asthma.[28]

Rafferty and Holgate[29] have shown that, in asthmatic subjects, orally administered terfenadine is a highly effective antagonist of histamine-induced bronchoconstriction, the maximal effect being observed using a 180 mg dose administered 3 h before the challenge. An identical effect on bronchoconstriction has been demonstrated using AMP as the inducer.[30-32] Recent work has suggested that the bronchoconstrictor effect of adenosine depends exclusively on the release of preformed mediators, rather than on newly generated mediators.[33] This explains the very similar bronchoconstrictor pattern produced by AMP and histamine, and the inhibitory effects of terfenadine.

In contrast, the bronchoconstrictor response to allergen challenge is attenuated, but not completely inhibited, by terfenadine, the overall response being reduced by 50%.[31, 34, 35] It appears, therefore, that although histamine release contributes significantly to the allergen-induced bronchconstriction it is not the only mediator involved and this effect must be also due to the contractile effects of lipid products (e.g. prostaglandin D_2, leukotriene C_4, platelet aggregation factor).

The effect of terfenadine on exercise-[36] and hyperventilation-induced asthma[37] has also been studied and it has been shown that the drug produces a significant, but not total, protection against both stimuli.[36]

Using a dose of terfenadine large enough to inhibit the histamine-induced bronchoconstriction, it has been confirmed that there is a treatment effect and that there is a significant difference between treatments in favour of terfenadine and, moreover, in the absence of period and carryover effects. Data for duration of distilled water challenge provide evidence that terfenadine provides more protection against bronchoconstriction induced by nebulized distilled water challenge than ipratropium bromide: for example, more patients were challenged with distilled water for a full 9 min in the presence of terfenadine than in the presence of ipratropium bromide (17 compared with 10 patients).

The finding that the mean pre-challenge FEV_1 for each treatment day was about 8% higher than for the control day suggests the possibility of bronchodilation following treatment with terfenadine or ipratropium bromide. Bronchodilation, however, was not assessed formally because the study was open in design and asthma is characterized by day-to-day baseline drift. In particular, FEV_1 was not measured before treatment on day 2 or 3. Although it was not assessed directly, bronchodilation was used as a covariate in the analysis of the bronchoconstrictor response to distilled water challenge in order to test for a relationship between bronchodilation and protection, that is whether bronchodilation improves protection. Analysis showed that protection against the bronchoconstrictor response was independent of any bronchodilation. The plot of bronchodilation versus distilled water-induced bronchoconstriction suggests, moreover, a negative relationship, that is bronchodilation impairs protection.

It is concluded that both histamine and cholinergic pathways appear to be involved in the asthmatic response to nebulized distilled water. Under the conditions of the present study, oral terfenadine was a more effective inhibitor of distilled water-induced bronchoconstriction than was inhaled ipratropium bromide. Protection was independent of bronchodilation, the magnitude of which was similar for the two treatments. Ultrasonically nebulized distilled water challenge may be a useful technique to study non-immunologically-induced bronchoconstriction in asthmatic patients.

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