

Inhibition of mast cell tryptase by inhaled APC 366 attenuates allergen-induced late-phase airway obstruction in asthma

Mamidipudi Thirumala Krishna, PhD, MRCP,^a Anoop Chauhan, PhD, MRCP,^a Louise Little, RN,^a Karen Sampson, RN,^a Richard Hawksworth, BSc,^b Tim Mant, MD,^b Ratko Djukanovic, MD, DM,^a Tak Lee, MD, FRCP,^c and Stephen Holgate, DSc, FRCP^a
Southampton and London, United Kingdom

Background: APC 366, a selective inhibitor of mast cell tryptase, has been shown to inhibit antigen-induced early asthmatic response (EAR), late asthmatic response (LAR), and bronchial hyperresponsiveness (BHR) in a sheep model of allergic asthma.

Objective: The purpose of this study was to investigate the effects of APC 366 on antigen-induced EAR, LAR, and BHR in mild atopic asthmatics not on any anti-inflammatory therapy.

Methods: Sixteen mild atopic asthmatics, each with a demonstrable antigen-induced EAR, LAR, and BHR to histamine, were recruited into this randomized, double-blinded, crossover study. APC 366 (5 mg)/placebo was administered by aerosol inhalation 3 times per day on treatment days 1 through 4. Allergen challenge was carried out on day 4. Histamine challenge was performed the following morning, 1 hour after final dosing.

Results: Subjects were shown to have a significantly smaller overall mean area under the curve for the LAR ($P = .012$) and mean maximum fall in FEV₁ for the LAR ($P = .007$) after pre-treatment with APC 366 in comparison with placebo. No significant effects on BHR were demonstrable. Although the EAR was reduced by 18% after treatment with APC 366 in comparison with placebo, this was not statistically significant.

Conclusion: Short-term repeated administration of APC 366 significantly reduced the magnitude of antigen-induced LAR in atopic asthmatics, which supports the role of mast cell tryptase in the pathophysiology of the LAR. (*J Allergy Clin Immunol* 2001;107:1039-45.)

Key words: APC 366, late asthmatic response, tryptase, asthma, mast cell, airway inflammation, bronchial hyperresponsiveness, early asthmatic response

Asthma is a chronic inflammatory disorder of the lower airways characterized by reversible airway obstruction and

Abbreviations used

AUC:	Area under the curve
BHR:	Bronchial hyperresponsiveness
EAR:	Early asthmatic response
FEV ₁ :	Forced expiratory volume in 1 second
IL:	Interleukin
LAR:	Late asthmatic response
PD ₂₀ :	Provocative dose to achieve a drop in FEV ₁ by 20% of postsaline baseline

mucosal inflammation. Bronchial challenge of atopic asthmatics with allergen results in an early phase—and in a proportion of subjects, a late phase—of bronchoconstriction that is associated with bronchial hyperresponsiveness (BHR). These effects are mediated through the IgE-dependent secretion of preformed and newly generated mediators from airway mast cells, and this has proved helpful in assessing the potential of anti-asthma drugs.^{1,2} The early asthmatic response (EAR) can largely be accounted for by the combined actions of histamine, prostaglandin D₂, and cysteinyl leukotrienes on airway smooth muscle.² Upregulation of leukocyte endothelial adhesion molecules—specifically, P-selectin, E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1—by mast cell mediators and inflammatory cytokines, in association with chemokine release, provides a mechanism for the influx of neutrophils and eosinophils accompanying the late asthmatic response (LAR).² Cysteinyl leukotrienes and platelet activation factor antagonists either have no effect or only partially attenuate the LAR when used alone or in combination with antihistamines.³⁻⁵ In addition, activated lung mast cells release large quantities of the unique 134-kd, 4 chained neutral protease, tryptase complexed to heparin.⁶ Bronchoalveolar lavage has shown high levels of tryptase in the airways of both adults⁷ and children⁸ with asthma, the levels rising markedly on exposure to inhaled allergen.⁹

Recent *in vitro* studies have suggested that tryptase could play a key role in the pathophysiology of the LAR.¹⁰⁻¹² Instillation of human tryptase into sheep airways caused both bronchoconstriction and BHR.¹³ In isolated dog¹⁴ and human¹⁵ airways, tryptase increases the contractility of smooth muscle to other mediators and is an important tissue kallikrein for generating bradykinin.¹⁶

From ^aUniversity of Southampton, Southampton General Hospital; ^bGuy's Drug Research Unit, London; and ^cthe Department of Respiratory Medicine and Allergy, GKT School of Medicine, Guy's Hospital, London. This study was sponsored by Axy's Pharmaceutical Corporation, San Francisco, Calif.

Received for publication May 3, 2000; revised March 9, 2001; accepted for publication March 9, 2001.

Reprint requests: Dr M. T. Krishna, Medical Specialties (RCMB Division), Mail Point 810, Level D, Centre Block, Southampton General Hospital, Tremona Road, Southampton SO16 6YD, United Kingdom.

Copyright © 2001 by Mosby, Inc.

0091-6749/2001 \$35.00 + 0 1/83/115631

doi:10.1067/mai.2001.115631

In sheep naturally sensitized to *Ascaris suum*, the novel selective inhibitor of tryptase APC 366 [N-(1-hydroxy-2-naphthoyl)-L-arginyl-L-prolinamide] attenuates the EAR, the LAR, acquired BHR, and the accompanying influx of inflammatory leukocytes on aerosol challenge with an antigenic extract.¹⁷ It has been shown that APC 366 inactivates tryptase in a time-dependent and irreversible manner involving slow isomerization of the hydroxynaphthyl group of APC 366 followed by nucleophilic attack of a tryptase amino acid side chain to result in the formation of a nonhydrolyzable covalent adduct between inhibitor and enzyme.¹⁸

In the present study, we tested the hypothesis that mast cell tryptase contributes to allergen-provoked airway responses in asthma. To this effect, we studied whether APC 366 has an effect on the allergen-provoked EAR, on the LAR, and on the associated increase in BHR in a group of mild atopic asthmatics.

MATERIALS AND METHODS

Study design

In this randomized, double-blinded, placebo-controlled, 2-period crossover study, subjects were randomized to receive either APC 366 followed by placebo or vice versa. During each of the 2 treatment periods, every subject received the treatment 3 times per day at intervals of 6 hours for 4 consecutive days and 1 time on the fifth day. On day 4, after the 10th dose of medication, every subject underwent allergen challenge followed by repeated spirometry to document an EAR and an LAR. On day 5, after the 13th dose of study medication, histamine challenge was performed to assess BHR. For both treatment periods, all subjects were resident in the Clinical Study Unit from the day before dosing (day 0) until their pulmonary function had returned to normal following histamine challenge and a final assessment by a physician on day 5.

Sample size

The sample size was calculated at a 5% level of significance and 80% power from a previous unpublished pilot study to detect a reduction of at least 50% in the LAR (measured as the area under the curve [AUC]) in the APC 366 treatment period in comparison with 15% in the placebo dosing period. The variability was estimated from the pilot study and from previous published crossover studies of similar design.^{19,20}

Subjects

Sixteen nonsmoking subjects with mild atopic asthma (mean age $\pm$ SE, 27.3 $\pm$ 1.6 years; 13 males and 3 females) with asthma that was well controlled without the use of systemic or inhaled corticosteroids were recruited into the study. A written informed consent was obtained from every subject, and the study was approved by the local ethics committee. Every subject underwent a detailed medical examination by a physician on screening day 1 and had PC₂₀ histamine of <16 mg/mL. Subjects with significant illness or abnormalities in routine hematologic and biochemical blood testing or urine examination were excluded. Only those female subjects who were postmenopausal, had been surgically sterilized, or were using adequate contraception and had negative pregnancy tests at screening and on admission to both treatment periods were recruited. None of the subjects had a history of respiratory infection before screening or before the study periods. In addition, the following medications were withheld for 6, 24, 24, 12, 24, 48, 24, 24, and 48 hours, respectively, before screening and treatment: inhaled short-acting β_2 ago-

nists, inhaled long-acting β_2 agonists, oral β_2 agonists, ipratropium bromide, oxitropium bromide, slow-release methyl xanthines, standard methyl xanthines, cromolyn, and nedocromil. Allergen and histamine challenges were performed on screening days 2 and 3, respectively. All subjects had (1) a clearly documented EAR, defined as a decrease in FEV₁ of at least 25% in comparison with postsaline baseline within 15 minutes after allergen inhalation and (2) an LAR, defined as a decrease in FEV₁ of at least 15% in comparison with baseline occurring between 3 and 9 hours after allergen inhalation. After allergen challenge, all subjects had a ≥ 2 -fold reduction in histamine PD₂₀ in comparison with their pre-allergen challenge values.

Skin prick testing and histamine challenge

Subjects underwent skin prick testing to common aeroallergens, including grass pollen, *Dermatophagoides pteronyssinus*, house dust, cat dander, and horse dander (Acquagen allergen, ALK, Berkshire, United Kingdom) through use of a standard protocol, as described previously.²¹ Only those subjects with at least 1 positive test (wheal diameter $\geq 5 \times 5$ mm) were included. Histamine challenges were performed through use of the modified dosimeter method of Chai, as described previously.^{22,23}

Allergen challenge

The allergen to which the patient demonstrated the strongest reaction in skin prick testing was used for bronchial challenge. Allergen was prepared in 10-fold dilutions with normal saline solution. Inhaled solutions were administered by means of the Spira Elektro breath-actuated inhalation dosimeter (Spira Respiratory Care Center, Hämeenlinna, Finland), which was set to a 0.6-second duration of delivery per actuation and driven by compressed air at a flow of 8 L/min. At each time point, 2 technically satisfactory measurements of FEV₁ were made; the higher of the 2 was recorded. With a nose clip in position, each subject inhaled 5 breaths of nebulized normal saline solution from functional residual capacity to total lung capacity, taking each inspiration over 5 seconds; this was followed by breath-holding for 2 seconds. Duplicate measurements of FEV₁ were made at 5 and 10 minutes after saline inhalation. The lesser of the best values at the 2 time points was taken as the post-saline baseline measurement.

Allergen inhalation was undertaken only if (1) FEV₁ was $\geq 70\%$ of the predicted value and (2) the postsaline fall in FEV₁ was $<10\%$. Allergen provocation was started with the top dilution (10^{-6}) of the allergen, and FEV₁ was recorded at 5, 10, and 15 minutes after inhalation. The lowest of the best values from each of the 3 time points was used to determine the drop in FEV₁ in comparison with postsaline baseline. If the fall was $<10\%$, the subject inhaled 5 breaths of the next higher concentration of the allergen. If the fall was between 10% and 15% of postsaline baseline, then the next dose of allergen that was administered was 5-fold, not 10-fold, higher. However, if the fall in FEV₁ was from 15% to 25% of postsaline baseline, an additional 5 breaths of the same concentration were taken. The challenge was terminated when a fall of $>25\%$ from the postsaline value was achieved. After this, FEV₁ recordings were made at 30, 60, 90, and 120 minutes relative to the last inhalation of allergen; this was followed by hourly intervals up to 9 hours after allergen inhalation. The dosage of allergen used after administration of APC 366/placebo was the same as that used at screening.

Study medication, dosage, and administration

Five milligrams of APC 366, dissolved in a 2-mL volume of 2.5% glycerol in 0.02 mol sodium phosphate buffer at pH 5.0, was administered by aerosol inhalation through use of a compressed air

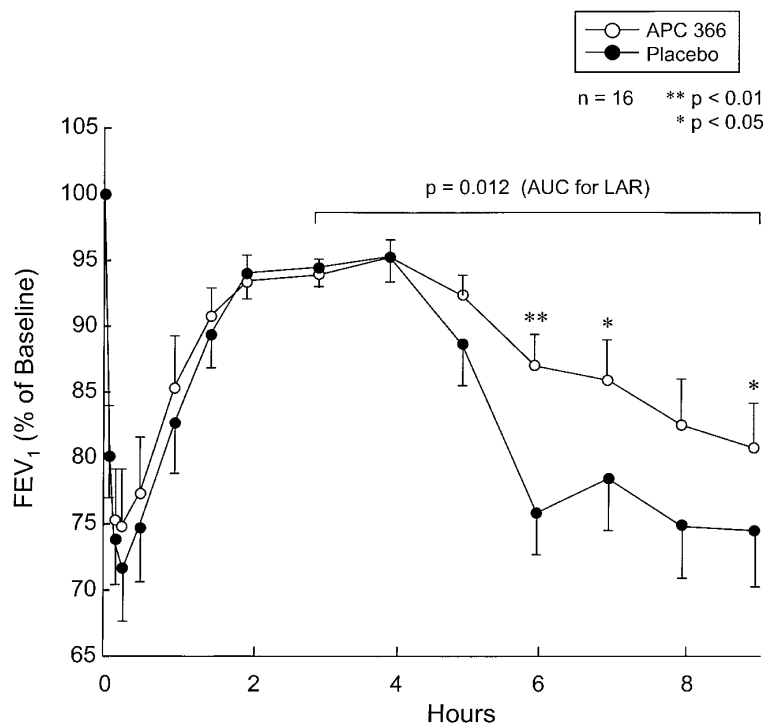


FIG 1. Effect of APC 366 and placebo on allergen-induced dual asthmatic responses. Significant differences ($P = .012$) were seen for AUC (for LAR) between APC 366 and placebo. In addition, comparison of mean percentage change in FEV₁ from baseline during LAR at respective time points between the 2 study days showed significant differences at 6, 7, and 9 hours, as shown by asterisk ($P < .05$) and double asterisk ($P < .01$).

nebulizer (Pari LC Jet, Pari Respiratory Equipment, Inc, Starnberg, Germany; mass median diameter, $<6 \mu\text{m}$), whereas solvent alone was used as placebo. Each subject inhaled the study medication by tidal breathing through a mouthpiece with a nose clip in place. Vital signs, including blood pressure, heart rate, and respiratory rate, were recorded on admission, immediately before, and 1 hour after the first drug dosing of each study day and before discharge. FEV₁ was recorded at baseline, before dosing, and 30 minutes after dosing, except after allergen challenge, when measurement of FEV₁ was performed according to the protocol, as described earlier. Laboratory analysis, including blood chemistry, hematology, coagulation, urine analysis, and pregnancy testing, was performed at screening, on day 0, and on day 5 (before discharge).

Statistical analyses

FEV₁ recorded during allergen challenge was expressed as a percentage change from postsaline baseline and plotted over time. The AUC for each subject was then calculated through use of the trapezoid rule between 0 and 120 minutes for the EAR and from 3 to 9 hours for the LAR for each treatment period. The mean maximum fall in FEV₁ (calculated from the maximum fall in FEV₁ during the EAR and the LAR for subjects during each treatment period) from the postsaline baseline value was derived within the time periods (0-120 minutes and 3-9 hours) and then compared between the 2 treatment periods. The dose of histamine that provoked a 20% fall in FEV₁ from baseline (PD₂₀) was used as a measure of BHR. PD₂₀ was calculated through use of the following formula: $\text{PD}_{20} = \text{antilog} \{ \log D_1 + [(\log D_2 - \log D_1) \times (20 - R_1) / (R_2 - R_1)] \}$, where D_1 is the cumulative dose up to and including the second-last dose ($<20\%$ FEV₁ fall), D_2 is the cumulative dose up to and including the last dose ($>20\%$ FEV₁ fall), R_1 is the percentage fall in FEV₁

after the second-last dose, and R_2 is the percentage fall in FEV₁ after the last dose. APC 366 and placebo were compared with respect to PD₂₀.

All of the measures described were analyzed through use of the Armitage and Berry²⁴ approach for crossover designs, which considers possible period effects when treatment effects are being investigated. A paired t test was used to compare the mean percentage changes in FEV₁ from baseline at respective time points (between APC 366 and placebo) during the LAR. A probability of $<.05$ was considered significant.

RESULTS

Allergen provocation caused both early- and late-phase falls in FEV₁ after both active and placebo treatments (Fig 1). The mean $\pm$ SEM values for maximum fall in FEV₁ during the EAR after APC 366 and placebo were 1.052 ± 0.209 (lit) and 1.183 ± 0.157 (lit), respectively; these were not significantly different. Moreover, no significant differences were seen in AUC_{EAR} between the 2 treatments [-1551.2 ± 387.86 vs -1890.6 ± 319.85 for APC 366 vs placebo [mean $\pm$ SEM]; Table I and Fig 1).

The mean $\pm$ SEM values for maximum fall in FEV₁ during the LAR after APC 366 and placebo were 0.786 ± 0.122 (lit) and 0.989 ± 0.124 (lit), respectively; these were significantly different ($P = .007$). Similarly, when the LAR was described as the AUC, the response after APC 366 was significantly less than the response after placebo (-3897.2 ± 717.94 vs -5856 ± 957.86 for APC 366 vs placebo [mean $\pm$ SEM]; $P = .012$; Table I and Fig 1).

TABLE I. Comparison of AUC, BHR, and mean maximum fall in FEV₁ following allergen challenges between APC 366 and placebo

	n	Placebo*	APC 366*	Treatment difference†	P value‡ (95% CI)
AUC for EAR	16	-1890.6 (319.85)	-1551.2 (387.86)	339.4	0.233 (-244.4, 923.1)
Mean maximum fall in FEV ₁ during EAR (L)	16	1.183 (0.157)	1.052 (0.209)	-0.131	0.456 (-0.496, 0.235)
AUC for LAR	16	-5856 (957.86)	-3897.2 (717.94)	1958.7	0.012 (502.4, 3415)
Mean maximum fall in FEV ₁ during LAR (L)	16	0.989 (0.124)	0.786 (0.122)	-0.204	0.007 (-0.342, -0.066)
PD ₂₀ histamine (μmoles)	14	0.277 (0.096)	0.171 (0.036)	-0.106	0.186 (-0.296, 0.064)

*All values are means ± SEMs.

†Treatment difference = APC - placebo, unadjusted for period effect.

‡Adjusted for period effect; P value is for treatment effect through use of Armitage and Berry approach for crossover designs. Although mean PD₂₀ following APC 366 appears lower, this was not statistically significant.**TABLE II.** Summary of data on physiologic measurements and allergens used for bronchial challenges

Subject no.	Allergen	Allergen conc to induce EAR at screening SQ* (U/mL)	Baseline FEV ₁ (lit)			PD ₂₀ histamine baseline (screening) μmol	PD ₂₀ histamine post-allergen challenge (μmol)			Time point for maximum fall in FEV ₁ for LAR (h)	
			Screening day	Placebo period	APC 366 period		Screening	APC 366	Placebo	Placebo	APC 366
1	HDM	1,000	3.45	3.88	3.27	0.29	0.07	0.043	0.055	9	6
2	Cat	100,000	6.07	5.83	5.56	1.72	0.42	0.407	0.954	6	3
3	HDM	100,000	4.15	3.83	3.85	0.66	0.34	0.221	0.399	9	8
4	GP	10,000	4.33	4.06	4.42	0.63	0.06	0.034	0.100	6	8
5	HDM	10,000	2.97	2.88	2.92	0.43	0.19	0.301	0.589	5	4
6	HDM	500	2.42	2.46	2.52	0.03	0.01	0.171	—	7	9
7	HDM	5,000	3.25	2.87	3.23	0.89	0.03	0.113	—	6	8
8	Cat	10,000	4.04	4.15	4.0	0.11	0.04	0.011	0.026	6	9
9	HDM	5,000 × 4†	3.84	3.58	3.40	0.20	0.09	0.032	0.065	8	9
10	HDM	100,000	4.49	4.44	4.62	0.20	0.06	0.335	0.119	8	8
11	HDM	10,000 × 2†	4.57	4.45	4.69	0.62	0.08	0.275	0.051	7	9
12	GP	100 × 2†	3.11	3.37	3.11	0.05	0.01	0.071	0.029	9	8
13	GP	1,000	3.53	3.70	3.46	0.36	0.03	0.032	0.018	9	9
14	HDM	10,000	3.0	3.20	3.25	0.76	0.12	0.150	0.208	8	7
15	GP	1,000	4.52	4.79	4.70	0.78	0.03	0.214	1.113	9	7
16	HDM	10,000	3.30	3.38	3.29	0.11	0.03	0.264	0.150	7	9
Mean (SD)			3.82 (0.88)	3.80 (0.83)	3.77 (0.82)	0.49 (0.43)	0.10 (0.12)	0.167 (0.126)	0.277 (0.360)		

*Allergen challenge protocols used for each subject following treatment with APC 366 and placebo were identical to screening.

†If fall in FEV₁ was between 15% and 25% of postsaline baseline, a further 5 breaths of the same concentration were used.

conc, Concentration; HDM, house dust mite; GP, grass pollen.

No significant differences were detectable in allergen-induced BHR to histamine between the 2 treatment periods (0.171 [0.036] vs 0.277 [0.096] for APC vs placebo [μmol histamine; mean ± SEM]).

With respect to mean percentage change in FEV₁, comparison between respective time points for the LAR revealed significant differences (placebo vs APC 366; mean [±SD]) at 6 hours (-22.58 [±13.02] vs -12.70 [±9.05]; *P* = .001), 7 hours (-20.76 [±15.19] vs -13.13 [±11.42]; *P* = .02), and 9 hours (-22.54 [±14.85] vs -16.52 [±14.52]; *P* = .02), with a trend at 8 hours (-21.55 [±14.60] vs -16.46 [±13.73]; *P* = .06). Data on allergens, PD₂₀, baseline FEV₁ at screening and before allergen challenge during study periods, and time points at which maximum fall in FEV₁ occurred during the LAR during both study periods are summarized in Table II.

DISCUSSION

This study has highlighted the contribution of mast cell tryptase to late-phase allergic responses in asthmatics after challenge with inhaled allergen. Short-term administration (by inhalation) of APC 366, a selective tryptase inhibitor, significantly attenuated the LAR by 33% and had a significant effect on the mean maximum fall in FEV₁ during the LAR. However, no significant effects were demonstrable on the allergen-induced EAR and BHR. These findings suggest that mast cell tryptase, through its recently identified proinflammatory properties involving protease activated receptors, could be contributing to the genesis and modulation of the LAR after allergen challenge in sensitized asthmatics.

The allergen challenge model of asthma has been used extensively to evaluate the efficacy of several anti-asthma

drugs, including inhaled corticosteroids,²⁵ long-acting β_2 -agonists,²⁶ sodium cromoglycate,²⁵ platelet activation factor,⁵ leukotriene receptor antagonists,⁴ and H_1 -antihistamines.^{3,4} In the present study, we selected (1) sensitized mild atopic asthmatics treated with β_2 -agonists only with significant baseline BHR to histamine and (2) subjects who exhibited dual asthmatic responses after challenge with allergen. In addition, all subjects had at least a 2-fold reduction in PD₂₀ to histamine 24 hours after allergen inhalation. These criteria enabled us to study the effects of APC 366 on the EAR, the LAR, and BHR after allergen challenge.

An 18% reduction in the EAR was detectable after treatment with APC 366, and this did not achieve statistical significance, although in the sheep model of asthma described earlier the allergen-provoked EAR was significantly attenuated.¹⁷ In human asthma, the EAR is known to be mediated by mast cell products, including histamine, sulfidopeptide leukotrienes (LTC₄, D₄, E₄), and PGD₂.² In light of the different mediators contributing to the development of the EAR after inhalation of allergen, one plausible mechanism by which APC 366 could have affected this response is its decreasing of the responsiveness of airway smooth muscle to mast cell tryptase. Recent studies have shown that tryptase can increase responsiveness of airway smooth muscle in vitro¹⁴ and on inhalation can induce bronchoconstriction in a sheep model of asthma,¹³ and indeed this response was abrogated with prior treatment with APC 366.¹³ One likely explanation for the failure to see a statistically significant attenuation in the EAR after administration of APC 366 is that this study was not sufficiently powered to detect a smaller response. Another possibility is that the EAR is mediated by other mediators alluded to earlier and the inhibition of tryptase alone does not influence the secretion or activity of these other mediators. Two recent studies^{27,28} with anti-IL-5 and recombinant human IL-12 in mild asthmatics in which the allergen challenge model was used did not show any effects on the EAR, the LAR, or BHR, although they did show significant effects on these endpoints in animal models (only on the EAR and the LAR—not on BHR with anti-IL-5). One of the explanations discussed by the authors in the anti-IL-5 study is that previous studies in human allergic asthma have shown dissociation among airway inflammation, BHR, and the LAR.^{29,30}

The significant attenuation of the LAR by APC 366 warrants further discussion. Neutrophils and eosinophils are known to play a key role in the genesis of the LAR,¹ and recent studies have shown that mast cell tryptase could induce leukocyte accumulation by a range of different mechanisms involving interaction of this proteinase with various substrates and cell types. He et al¹² have recently shown that injection of low concentrations of purified tryptase in guinea pig skin induces an ingress of large numbers of eosinophils and neutrophils close to the injection site.¹² This response was dose-dependent, and some of the eosinophils were degranulated.¹² Mast cell tryptase could contribute to granulocyte influx

through several mechanisms. Cleavage of complement component C3 by tryptase results in the formation of anaphylotoxin C3a, a potent chemoattractant for neutrophils.³¹ In another recent in vitro study, mast cell tryptase has been shown to be a mitogen for epithelial cells and to stimulate IL-8 production and intercellular adhesion molecule-1 expression; both of these factors are known to play a key role in the recruitment of eosinophils and neutrophils.¹⁰ More recently, Compton et al¹¹ have shown that tryptase can induce in human umbilical vein endothelial cells the production of IL-8, a chemokine established as a potent chemoattractant and activator of neutrophils as well as eosinophils. Tryptase itself is a chemoattractant in Boyden chambers for eosinophils and is capable of inducing release of eosinophil cationic protein from eosinophils.³² These studies provide plausible mechanisms for the biological actions of mast cell tryptase in the LAR, and future studies should address the anti-inflammatory action of APC 366 in vivo in allergic asthmatics after allergen challenge. In the absence of data showing changes in allergen-induced inflammation after treatment with APC 366, it is not possible to categorically attribute the attenuation of the LAR seen in this study to an anti-inflammatory effect of APC 366; assessment of this by induced sputum, bronchoalveolar lavage, and mucosal biopsy would be worth pursuing. Nevertheless, our results are compatible with previous findings in a sheep model of asthma, and they support this concept.

Although it has been shown that eosinophils and neutrophils play a pivotal role in the LAR, recent studies with zafirlukast, a leukotriene receptor antagonist, and the antihistamine loratadine have suggested that cysteinyl leukotrienes and histamine could play a major role in the genesis of the LAR after challenge with allergen, because combined treatment with zafirlukast and loratadine attenuated the LAR by 75%.⁴ It follows that inhibition of mast cell tryptase might not have any effect on the release of histamine and cysteinyl leukotrienes, and this could explain why treatment with APC 366 in the present study attenuated the LAR by only 33%.

Another interesting observation made in this study is the absence of any effect of APC 366 on allergen-induced increase in BHR. Given the general hypothesis of the association between the LAR and allergen-induced BHR,³³ this observation is surprising and difficult to explain. Nevertheless, 3 plausible explanations of the discrepancy can be considered. First, few studies have reported the development of BHR soon after the EAR, suggesting that the LAR is not necessarily a prerequisite for the increase in BHR after challenge with allergen, although a dual response would most likely serve to enhance the magnitude of change in BHR.^{34,35} Second, in the present study APC 366 failed to suppress the EAR significantly and only attenuated the LAR by 33%, raising the possibility of separate mechanisms contributing to BHR that are independent of tryptase. Third, the dose of APC 366 used in this study might not have been sufficient to affect the EAR and BHR, inasmuch as the sheep study¹⁷ showed a dose-response relationship. We chose

the present dosing regime on the basis of our previous observations in phase I and IIa studies, which have shown that this regimen is nontoxic and can be well tolerated.

The molecular mechanism(s) by which tryptase could produce effects pertinent to the inflammatory and remodeling responses in asthma has received added impetus since the discovery of a series of tethered ligand receptors that are activated by proteases.³⁶ Tryptase is known to activate protease activated receptor-2, and a significant proportion of this response can be reproduced by the peptide SLIGKV, which activates the G-protein mechanism by replacing the amino acid sequence exposed on cleavage of protease activated receptor-2.³⁷ In this regard, the chemotactic and cell activation properties of human tryptase on eosinophils is of specific interest in relation to the LAR.¹²

The present study demonstrates the potential role of APC 366 in the management of allergic disease, including asthma, allergic rhinitis, and allergic conjunctivitis. Recent reports suggest that human mast cell tryptase can stimulate collagen synthesis in the human lung fibroblast cell line and can have both direct and indirect effects on connective tissue metabolism.³⁸ Thus, tryptase inhibitors might be useful for treating fibrotic diseases such as idiopathic pulmonary fibrosis and scleroderma, in which mast cell involvement has been noted.

In conclusion, this study has shown that APC 366, a selective tryptase inhibitor, partially inhibits the allergen-induced LAR in mild atopic asthmatics; our findings support a role for mast cell tryptase in this response.

We express our thanks to all Clinical Research Fellows in Southampton who provided night medical cover during the study; to the research nurses for their help with patient care, drug administration, and challenges at both centers; to F. Anderson and Dr Dik Ng for help with administration; and to all of the patients without whose participation this study would not have been possible.

REFERENCES

- Bentley AM, Kay AB, Durham SR. Human late asthmatic reactions. *Clin Exp Allergy* 1997;27:71-86.
- Galli SJ, Costa JJ. Mast cell-leukocyte cytokine cascades in allergic inflammation. *Allergy* 1995;50:851-62.
- Twentyman OP, Ollier S, Holgate ST. The effect of H₁-receptor blockade on the development of early- and late-phase bronchoconstriction and increased bronchial responsiveness in allergen-induced asthma. *J Allergy Clin Immunol* 1993;91:1169-78.
- Roquet A, Dahlén B, Kumlin M, Ihre E, Anstrén G, Binks S, et al. Combined antagonism of leukotrienes and histamine produces predominant inhibition of allergen-induced early and late phase airway obstruction in asthmatics. *Am J Respir Crit Care Med* 1997;155:1856-63.
- Arima M, Yukawa J, Makino S. Effect of YM264 on the airway responsiveness and late asthmatic response in a guinea pig model of asthma. *Chest* 1995;108:529-34.
- Walls AF. Mast cell protease in asthma. In: Holgate ST, Busse W, editors. *Inflammatory mechanisms in asthma*. New York: Marcel Dekker;1998. p. 89-110.
- Broide DH, Gleich GJ, Cuomo AJ, Cuburn DA, Federman DC, Schwartz LB, et al. Evidence of ongoing mast cell and eosinophil degranulation in symptomatic asthma airway. *J Allergy Clin Immunol* 1991;88:637-48.
- Ferguson AC, Whitelaw M, Brown H. Correlation of bronchial eosinophil and mast cell activation with bronchial hyperresponsiveness in children with asthma. *J Allergy Clin Immunol* 1992;90:609-13.
- Wenzel SE, Fowler AA, Schwartz LB. Activation of pulmonary mast cells by bronchoalveolar lavage allergen challenge. In vivo release of histamine and tryptase in atopic and nonatopic subjects with and without asthma. *Am Rev Respir Dis* 1988;137:1002-8.
- Cairns JA, Walls AF. Mast cell tryptase is a mitogen for epithelial cells: stimulation of IL-8 production and intercellular adhesion molecule-1 expression. *J Immunol* 1996;156:275.
- Compton SJ, Cairns JA, Holgate ST, Walls AF. The role of mast cell tryptase in regulating endothelial cell proliferation, cytokine release and adhesion molecule expression. Tryptase induces expression of mRNA for IL- β and IL-8 and stimulates the selective release of IL-8 from HUVEC. *J Immunol* 1998;161:1939-46.
- He SH, Peng Q, Walls AF. Potent induction of a neutrophil and eosinophil-rich infiltrate in vivo by human mast cell tryptase: selective enhancement of eosinophil recruitment by histamine. *J Immunol* 1997;159:6216-25.
- Molinari JF, Scuri M, Moore WR, Clark J, Tanaka R, Abraham WM. Inhaled tryptase causes bronchoconstriction in sheep via histamine release. *Am J Respir Crit Care Med* 1996;154:649-53.
- Sekizawa K, Caughey GH, Lazarus SC, Gold WM, Nadel JA. Mast cell tryptase causes airway smooth muscle hyperresponsiveness in dogs. *J Clin Invest* 1989;83:175-9.
- Johnson PRA, Ammit AJ, Carlin SM, Armour CL, Caughey GH, Black JL. Mast cell tryptase potentiates histamine-induced contraction of human sensitized bronchus. *Eur Respir J* 1997;10:38-48.
- Imamura T, Dubin A, Moore W, Tanaka R, Travis J. Induction of vascular permeability enhancement by tryptase: dependence on activation of prekallikrein and direct release of bradykinin from kininogens. *Lab Invest* 1996;74:861-70.
- Clark JM, Abraham WM, Fishman CE, Forteza R, Ahmed A, Corter A, et al. Tryptase inhibitors block allergen-induced airway and inflammatory responses in allergic sheep. *Am J Respir Crit Care Med* 1995;152:2076-83.
- Clark JM, Moore WR, Tanaka RD. Tryptase inhibitors: a new class of antiinflammatory drugs. *Drugs of the Future* 1996;21:811-6.
- Taylor IK, O'Shaughnessy KM, Fuller RW, Dollery CT. Effect of cyteinyl-leukotriene receptor antagonist ICI 204,219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. *Lancet* 1991;337:690-4.
- Friedman BS, Bel EH, Buntix A, Tanaka W, Han YHR, Shingo S, et al. Oral leukotriene inhibitor (MK-886) blocks allergen-induced airway responses. *Am Rev Respir Dis* 1993;147:839-44.
- Pepys J. Skin testing. *Br J Hosp Med* 1975;14:412-21.
- Djukanovic R, Wilson JW, Lai CKW, Holgate ST, Howarth PHH. The safety aspects of fiberoptic bronchoscopy, bronchoalveolar lavage and endobronchial biopsy in asthma. *Am Rev Respir Dis* 1991;143:772-7.
- Chai H. Antigen and methacholine challenge in children with asthma. *J Allergy Clin Immunol* 1979;64(6 pt 2):575-9.
- Armitage P, Berry G. *Statistical methods in medical research*. 2nd ed. Oxford: Blackwell Scientific Publishers; p. 120-3.
- Cockcroft DW, Murdock KY. Comparative effects of inhaled salbutamol, sodium cromoglycate, and beclomethasone propionate on allergen-induced early asthmatic responses, late asthmatic responses, and increased bronchial responsiveness to histamine. *J Allergy Clin Immunol* 1987;79:734-40.
- Weersink EJM, Aalbers R, Koeter GH, Kauffman HF, Demonchy JGR, Postma DS. Partial inhibition of the early and late asthmatic response by a single dose of Salmeterol. *Am J Respir Crit Care Med* 1994;150:1262-7.
- Bryan SA, O'Connor BJ, Matti S, Leckie MJ, Kanabar V, Khan J, et al. Effects of recombinant human IL-12 on eosinophils, airway hyperresponsiveness, and the late asthmatic response. *Lancet* 2000;356:2149-53.
- Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-8.
- Crimi E, Spanvello A, Neri M, Ind PW, Rossi GA, Brusasco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. *Am J Respir Crit Care Med* 1998;157:4-9.
- Rosi E, Ronchi MC, Grazzini M, Duranti R, Scano G. Sputum analysis, bronchial hyperresponsiveness, and airway function in asthma: results of a factor analysis. *J Allergy Clin Immunol* 1999;103:232-7.
- Schwartz LB, Kawahara MS, Hugli TE, Vik D, Fearon DT, Austen KF. Generation of C3a anaphylatoxin from human C3 by human mast cell tryptase. *J Immunol* 1983;130:1891-5.
- Walls AF, He S, Teran LM, Buckley MG, Jung KS, Holgate ST, et al. Granulocyte recruitment of human mast cell tryptase. *Int Arch Allergy Appl Immunol* 1995;107:372-3.

33. Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in nonallergic bronchial reactivity. *Clin Allergy* 1977;7:503-13.
34. Thorpe JD, Steinberg D, Bernstein IL, Murlas CG. Bronchial reactivity increases soon after the immediate response in dual-responding asthmatic subjects. *Chest* 1987;91:21-5.
35. Bernstein DI, Ploysongsang Y, Mittman RJ, Piyamahunt A, Bernstein IL. The relationship between airway responsiveness measured before and after allergen-induced late asthmatic responses. *Chest* 1992;101:437-41.
36. Molino M, Barnathan ES, Numerof R, Clark J, Dreyer M, Cumashi A, et al. Interactions of mast cell tryptase with thrombin receptors and PAR-2. *J Biol Chem* 1997;272:4043-9.
37. Perng D-W, Holgate ST, Walls AF. Mast cell tryptase as a stimulus for cytokine production and release from epithelial cells: role for PAR-2. *Immunology* 1998;95:P21.
38. Cairns J, Walls AF. Mast cell tryptase stimulates the synthesis of type I collagen in human lung fibroblasts. *J Clin Invest* 1997;99:1313-21.

ON THE MOVE?

Send us your new address at least six weeks ahead

Don't miss a single issue of the journal! To ensure prompt service when you change your address, please photocopy and complete the form below.

Please send your change of address notification at least six weeks before your move to ensure continued service. We regret we cannot guarantee replacement of issues missed due to late notification.

JOURNAL TITLE:

Fill in the title of the journal here. _____

OLD ADDRESS:

Affix the address label from a recent issue of the journal here.

NEW ADDRESS:

Clearly print your new address here.

Name _____

Address _____

City/State/ZIP _____

COPY AND MAIL THIS FORM TO:

Mosby
Subscription Customer Service
6277 Sea Harbor Dr.
Orlando, FL 32887

OR FAX TO:

407-363-9661



OR PHONE:

800-654-2452
Outside the U.S., call
407-345-4000