

# The effect of marimastat, a metalloprotease inhibitor, on allergen-induced asthmatic hyper-reactivity

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## Abstract

This pilot study was designed to assess whether a synthetic matrix metalloproteinase (MMP) inhibitor has anti-inflammatory properties in mild asthma. Tumor necrosis factor alpha (TNF $\alpha$ ) has been shown to be an important cytokine in the pathogenesis of allergic airway inflammatory responses, and its release can be inhibited by MMP inhibitors.

Twelve atopic asthmatic subjects received the MMP inhibitor marimastat (5 mg) or placebo, twice daily for 3 weeks, separated by a 6-week washout period in a randomized, double-blind, cross-over manner. All subjects underwent an allergen inhalation provocation test to *Dermatophagoides pteronyssinus* before and after each study phase. Spirometry, exhaled NO (eNO) levels, differential sputum cell counts, an asthma symptom questionnaire, peak flow, and  $\beta_2$ -agonist usage were measured.

Nine subjects completed the study, and, when compared with placebo, marimastat reduced bronchial hyper-responsiveness to inhaled allergen in these subjects from an allergen PC<sub>20</sub> of 22.2 AU/ml (95%CI 11.7–32.6) to 17.0 AU/ml (95%CI 7.6–26.4,  $P = 0.02$ ). The marimastat phase showed a nonsignificant fall in sputum inflammatory cells. Marimastat did not modify eNO, FEV<sub>1</sub>, asthma symptoms, or albuterol usage.

In conclusion, airway responsiveness to allergen may be modified by a MMP inhibitor, perhaps via TNF $\alpha$  playing a role in airway inflammation and remodeling.

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**Keywords:** TNF $\alpha$ ; Asthma; Allergen; Marimastat

## Introduction

Tumor necrosis factor alpha (TNF $\alpha$ ) has been shown to be an important cytokine in the pathogenesis of allergic airway inflammatory responses (Casale et al., 1996; Thomas, 2001). Previous studies have shown that inhalation of TNF $\alpha$  increases airway responsiveness and neutrophil infiltration, in addition, TNF $\alpha$  is increased after allergen inhalation (Keatings et al., 1997; Thomas et al., 1995).

TNF $\alpha$  is released from a cell membrane-anchored precursor by proteolytic cleavage by a TNF $\alpha$  converting enzyme (TACE) or ADAM-17 (ADAM: proteins containing a disintegrin and metalloproteinase domain). In addition,

TNF $\alpha$  has been shown to be activated by a number of matrix metalloproteinases (MMPs) such as stromelysin, matrilysin, collagenase, and gelatinases A and B (Gearing et al., 1994). Gelatinase-B (MMP-9) levels are increased after an allergen challenge, and it has been implicated in inflammatory cellular migration and airway remodeling (Kelly et al., 2000; Lee et al., 2001). TNF $\alpha$  production can be reduced by inhibiting TACE via tissue inhibitor metalloproteinase-3 (TIMP-3) (Armour et al., 1998).

This study investigated whether a synthetic MMP inhibitor has anti-inflammatory properties in patients with asthma by reducing TNF $\alpha$  production in vivo via inhibition of MMP activity and hence TNF $\alpha$  release. It was postulated that this would reduce airway inflammatory responses and bronchial hyper-responsiveness that are commonly associated with elevated TNF $\alpha$  production after an allergen challenge.

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We investigated whether a broad spectrum MMP inhibitor such as marimastat (BB-2516, British Biotech, Oxford, UK) would modify the inflammatory response to allergen-induced bronchoconstriction and baseline control of mild asthma. Preclinical studies have shown that this group of MMP inhibitors is able to reduce the production of TNF $\alpha$  in vivo and marimastat has an effective oral dose range of 10–100 mg/day in man when used in studies for the treatment of neoplasia. The main side effects of this drug seen at doses above this range are fatigue and an inflammatory polyarthritis that is reversible on discontinuation of treatment (Gearing et al., 1994, 1995; Wojtowicz-Praga et al., 1998).

## Materials and methods

**Study subjects.** Twelve nonsmoking steroid naive mild asthmatic subjects (male/female 4/8, mean age 29.1 (SD  $\pm$  12) years) were recruited, and eligibility criteria included a forced expiratory volume in 1 s (FEV<sub>1</sub>) greater than 70% predicted, a positive skin prick test to *Dermatophagoides pteronyssinus* (*Der p*) (Bayer Corporation, Elkhart, IN, USA), a bronchial inhalation challenge that caused a 20% fall in baseline forced expiratory volume (FEV<sub>1</sub>) at a provocative concentration (PC<sub>20</sub>) of less than 8 mg/ml to methacholine (MCh) (Sigma, St. Louis, MO, USA) (American Thoracic Society, 1999a), and a repeatable exhaled nitric oxide measurement (Table 1). Skin prick testing was performed using a panel of 14 allergens, a positive and a negative control, according to the guidelines of the American Academy of Allergy, Asthma and Immunology and the American College of Allergy, Asthma and Immunology, where a response 3 mm greater than vehicle

control is considered positive (Bernstein and Storms, 1995). None had a more than 1 pack year history of smoking or smoking-related disease. Exclusion criteria were inability to perform breathing maneuvers, a recent upper respiratory tract infection, or requirement for any glucocorticosteroid. The study was approved by the institutional ethics committee and all subjects gave written, informed consent.

**Study design.** A pilot study was conducted to determine the effect of marimastat on allergen-induced hyper-reactivity. All subjects underwent an allergen challenge to *Der p* before and after treatment with either placebo or marimastat, and exhaled NO (eNO) and FEV<sub>1</sub> were monitored for 7 h. Sputum cell counts and methacholine challenge were also performed at the end of each allergen challenge days. During each treatment phase, daily peak expiratory flow (PEF), symptoms, and  $\beta_2$  adrenergic agonist usage were self-recorded. There was a washout period of 6 weeks between each treatment arm (Fig. 1).

**Methods.** Marimastat (5 mg) or placebo was given twice daily for 3 weeks separated by a 6-week washout period in a randomized double-blind, cross-over manner. Randomization was computer generated by British Biotech PLC, and phase allocation was concealed in sealed envelopes.

Allergen challenges were performed at the beginning and end of each treatment phase using standardized aqueous allergen extracts of *D. pteronyssinus* (30 000 AU/ml), delivered in a closed system by a hand-held nebulizer connected to a Hans-Rudolph non-rebreathing, three-way valve (Hans Rudolph Inc, Kansas City, MO, USA) (Melillo et al., 1991; Sterk et al., 1993).

The initial concentration for allergen inhalation was individually calculated to start four doubling dilutions below the predicted allergen PC<sub>20</sub>. Predicted allergen PC<sub>20</sub> was calculated by methacholine responsiveness and skin prick sensitivity at screening, as previously described by Crockcroft et al. (1987).

Doubling dilutions of aqueous *Der p* extract were diluted in phosphate-buffered saline (PBS) and inhaled for 2 min, and FEV<sub>1</sub> measured at 10-min intervals after each inhalation. The challenge was ceased when FEV<sub>1</sub> had fallen by >20% and the concentration of *Der p* in allergen units (AU)/ml that gave a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub>) was then calculated by interpolation.

Subjects received 200  $\mu$ g albuterol and FEV<sub>1</sub> was measured every 10 min for the first hour, at 90 min and 2 h, and then every hour for 7 h after the challenge using a dry bellows spirometer (Vitalograph, Buckingham, UK). Exhaled NO (eNO) was measured on-line every hour for 7 h after the allergen challenge using a modified chemiluminescence analyzer (Model 2107, Dasibi Environmental Corp. Glendale, CA), in accordance with current recommendations (American Thoracic Society, 1999b). At the conclusion of each allergen challenge day, differential cell counts in sputum was obtained via inhalation of 4.5%

Table 1  
Baseline characteristics of study subjects

| Subject         | Sex | Age<br>(years) | Baseline<br>FEV <sub>1</sub> %<br>predicted | MCh <sup>a</sup><br>PC <sub>20</sub><br>mg/ml | Predicted <sup>b</sup><br><i>Der p</i><br>PC <sub>20</sub><br>AU/ml |
|-----------------|-----|----------------|---------------------------------------------|-----------------------------------------------|---------------------------------------------------------------------|
| 1               | F   | 28             | 80.3                                        | 0.3                                           | 1.38                                                                |
| 2               | F   | 21             | 87.7                                        | 2.3                                           | 0.33                                                                |
| 3               | M   | 43             | 66.1                                        | 2.0                                           | 88.63                                                               |
| 4               | F   | 26             | 84.8                                        | 0.2                                           | 1.02                                                                |
| 5               | F   | 19             | 80.9                                        | 5.2                                           | 41.77                                                               |
| 6               | F   | 21             | 97.3                                        | 1.1                                           | 11.04                                                               |
| 7               | M   | 25             | 98.5                                        | 1.9                                           | 1.97                                                                |
| 8               | F   | 38             | 73.3                                        | 0.4                                           | 1.84                                                                |
| 9               | F   | 59             | 81.7                                        | 0.1                                           | 0.17                                                                |
| 10 <sup>c</sup> | M   | 23             | 72.8                                        | 0.9                                           | 1.89                                                                |
| 11 <sup>c</sup> | M   | 19             | 80.3                                        | 2.5                                           | 15.21                                                               |
| 12 <sup>c</sup> | F   | 27             | 78.1                                        | 0.1                                           | 4.51                                                                |
| Mean            |     | 29.1 years     | 81.8                                        | 1.4 mg/ml                                     | 14.2 AU/ml                                                          |
| 95%CI           |     | (22.3–35.8)    | (76.5–87.2)                                 | (0.6–2.2)                                     | (0.0–29.0)                                                          |

Baseline values are means with 95% confidence intervals, unless stated otherwise.

<sup>a</sup> Methacholine challenge.

<sup>b</sup> Allergen Units (30,000 AU/ml).

<sup>c</sup> Withdrawn from study.

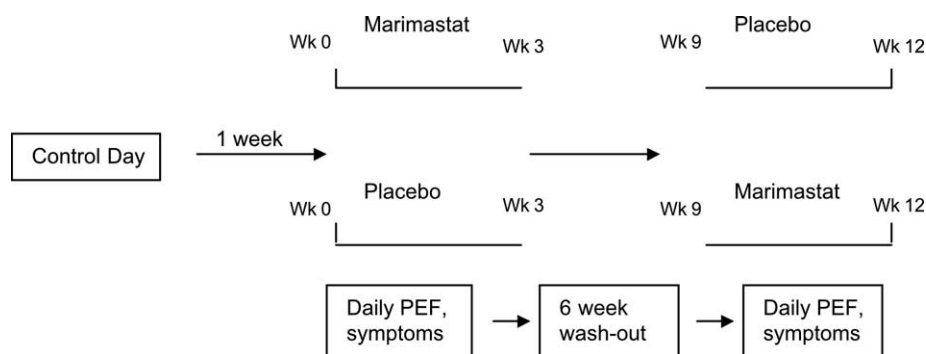


Fig. 1. Study protocol. The control day involved a skin prick test to *Der p*, methacholine challenge, and exhaled NO and FEV<sub>1</sub> were monitored for 7 h. At weeks 0, 3, 9, and 12, all subjects underwent allergen challenge to *Der p* before and after treatment with either placebo or marimastat, and eNO and FEV<sub>1</sub> were monitored for 7 h. During each treatment phase, daily PEF, symptoms, and  $\beta_2$  agonist usage were self-recorded. There was a washout period of 6 weeks between each treatment arm.

hypertonic saline via ultrasonic nebulizer (Ultra-Neb 2000, DeVibriss Health Care, Somerset, PA, USA) and incubated with 0.1% dithiothreitol (DTT) (Sigma), washed, and the cell pellet mounted on slides, fixed, and stained with hematoxylin and eosin (DiffQuick) (Fahy et al., 1993).

During the two phases, subjects also completed a previously validated twice daily asthma symptom questionnaire (Yates et al., 1996), monitored PEF with a personal peak-flow meter (Personal Best Peak Flow Meter HS755, Respironics, USA), and recorded  $\beta_2$ -agonist usage; in addition, weekly monitoring of hematology, liver, and renal function was also carried out. Compliance was assessed by end of phase residual capsule count.

**Analysis.** The primary outcome measure was a significant change in allergen PC<sub>20</sub>, at the end of each 3-week treatment period, with secondary outcomes of change in baseline FEV<sub>1</sub> and eNO, serial PEF measurements, asthma symptoms,  $\beta_2$ -agonist usage, and sputum inflammatory cells. Data were analyzed using SPSS version 8 (SPSS, Chicago, IL, USA). Both PC<sub>20</sub> and eNO were log-normally distributed as previously noted (Thomas et al., 1995; Yates et al., 1995). Comparisons of FEV<sub>1</sub> were analyzed by repeated measures two-way analysis of variance (ANOVA) and eNO by one-way ANOVA. Early allergen response (EAR) and late allergen response (LAR) were calculated from the change in baseline FEV<sub>1</sub> as area under the time-response curve (AUC) (Sterk et al., 1993). Paired *t* tests were used for allergen PC<sub>20</sub>, baseline FEV<sub>1</sub>, compliance, and peak flow measurements. Nonparametric data were analyzed using the Wilcoxon signed-rank test (differential count, symptoms, albuterol usage). Results are expressed as means unless stated otherwise, with 95% confidence intervals and a *P* value of less than 0.05 considered to indicate statistical significance.

**Ex vivo experiments.** In order to establish that the marimastat dosage was in the correct range for TNF inhibition, post hoc experiments were performed on peripheral blood monocytes (PBMC). Using a dose of marimastat 5 mg twice daily in normal individuals for 3

days, PBMCs were extracted before and after marimastat therapy and cultured in RPMI for 48 h, having removed macrophages by adhesion to plastic. TNF content of the supernatant with and without 100 ng/ml lipopolysaccharide (LPS) was determined by ELISA (R&D Systems).

## Results

Two subjects were withdrawn with respiratory tract infections, and one was noncompliant with the study schedule. Nine subjects successfully completed both phases of the study and the compliance rate was 88% and 89% during the marimastat and placebo phase, respectively. Data was analyzed on an intention to treat basis. No adverse outcomes were reported, and renal, liver function, and hematological tests remained normal.

No cross-over effects were observed. The FEV<sub>1</sub> and allergen PC<sub>20</sub> were not significantly different at the commencement of each phase.

All subjects exhibited bronchial hyper-reactivity to inhaled methacholine with a baseline geometric mean PC<sub>20</sub> methacholine of 1.4 mg/ml (95%CI 0.6–2.2). All had a positive skin prick test to *Der p*, from this and the methacholine PC<sub>20</sub> the predicted allergen PC<sub>20</sub> was calculated (Crockcroft et al., 1987). These features define the subjects as having mild, atopic asthma (American Thoracic Society, 1999a).

During the marimastat phase, subjects were able to inhale more allergen and the allergen-induced provocative concentration that induced a 20% fall in FEV<sub>1</sub> (allergen PC<sub>20</sub>) after treatment with marimastat was significantly increased when compared with placebo, 22.2 AU/ml (95%CI 11.7–32.6) compared with 17.0 AU/ml (95%CI 7.6–26.4, *P* = 0.02). There was a clear shift in the dose-response curve to the right at all concentrations, indicating a reduction in responsiveness (Fig. 2).

Mean baseline FEV<sub>1</sub> improved after the marimastat phase, from 2.83 L (95%CI 2.34–3.23) to 2.91 L (95%CI 2.40–3.43), but this was not significant when compared with

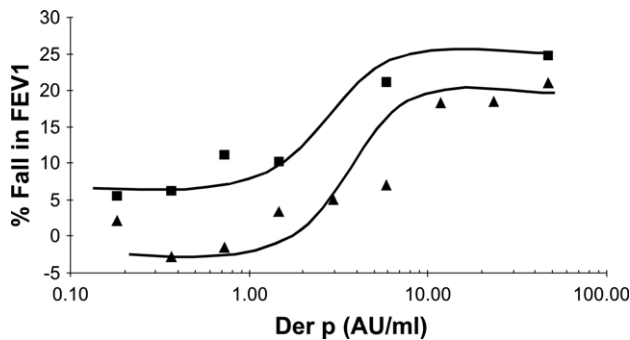


Fig. 2. Allergen log-dose response curves. Subjects underwent an inhalation challenge with house dust mite allergen to achieve a >20% fall in forced expiratory volume (FEV<sub>1</sub>). Results are expressed as log-dose response to inhaled *Der p* allergen (allergen units/ml) in the nine subjects who completed the study after treatment with marimastat (closed triangles) or placebo (closed squares). There was a significant shift in the dose response to the right when the control phase was compared with the marimastat phase. The allergen-induced provocative concentration that induced a 20% fall in FEV<sub>1</sub> (allergen PC<sub>20</sub>) after treatment with marimastat was significantly increased when compared with placebo, 22.2 AU/ml (95%CI 11.7–32.6) compared with 17.0 AU/ml (95%CI 7.6–26.4,  $P = 0.02$ ).

the mean baseline FEV<sub>1</sub> after the placebo phase. Airway responses to inhaled allergen during EAR (AUC<sub>0–3 h</sub>) were not significantly different between the two phases,  $1.0 \pm \text{SEM} 1.7\%$  during placebo and  $0.5 \pm 1.4\%$  during marima-

stat treatment (mean treatment difference, 0.5% (95%CI –2.3 to 3.2,  $P = 0.75$ ); however, the subjects had been given albuterol to reverse their initial bronchoconstriction. During the LAR (AUC<sub>3–7 h</sub>), the mean percent fall in FEV<sub>1</sub> per hour was significantly greater after marimastat compared to placebo (mean treatment difference 7.7% (95%CI 6.8 to 8.7,  $P = 0.03$ ) (Table 2).

The mean exhaled NO (eNO) over the 7 h of allergen challenge was significantly higher after marimastat, log(eNO ppb) 1.59 (95%CI 1.54–1.65), when compared with the placebo phase, log(eNO ppb) 1.47 (95%CI 1.37–1.57,  $P = 0.03$ ). At the end of the allergen challenge day, however, eNO was not significantly different between marimastat and placebo phase, despite the larger amount of allergen inhaled during the former. Total sputum inflammatory cells (eosinophils, neutrophils, and lymphocytes) were reduced by 3.3% (95%CI –15.1% to 8.4%) after treatment with marimastat, but did not reach significance (Wilcoxon,  $z = -1.2$ ,  $P = 0.23$ ). There were no significant differences in columnar cells or total number of macrophages between the two phases (Table 2).

Bronchial hyper-responsiveness to methacholine was reduced after marimastat when compared with placebo. Mean methacholine PC<sub>20</sub> was greater after the marimastat phase compared with the placebo, 0.8 mg/ml (95%CI 0.1–

Table 2  
Effect of marimastat or placebo in response to inhaled allergen in asthmatic subjects

|                                                                   | Placebo mean (95%CI)      | Marimastat mean (95%CI)    | $P$ value ( $n = 9$ ) |
|-------------------------------------------------------------------|---------------------------|----------------------------|-----------------------|
| PC <sub>20</sub> allergen (AU/ml) <sup>a</sup>                    | 17.0 AU/ml (7.6–26.5)     | 22.2 AU/ml (11.7–32.6)     | 0.02*                 |
| Baseline FEV <sub>1</sub>                                         | 2.83 (2.34–3.23)          | 2.91 (2.40–3.43)           | 0.32                  |
| LAR <sub>3–7 h</sub> (expressed as FEV <sub>1</sub> % fall per h) | 9.7% (4.6–14.9)           | 17.4% (10.1–24.6)          | 0.03*                 |
| Total mean log exhaled NO (ppb)                                   | 1.47 (1.37–1.57)          | 1.59 (1.54–1.65)           | 0.03*                 |
| <i>Mean differential sputum cell count</i>                        |                           |                            |                       |
| Macrophages                                                       | 91.8% (367/400)           | 92.8% (371/400)            | 0.69                  |
| Neutrophils                                                       | 3.0% (12/400)             | 2.0% (8/400)               | 0.19                  |
| Lymphocytes                                                       | 2.8% (11/400)             | 3.0% (12/400)              | 0.89                  |
| Eosinophils                                                       | 1.2% (5/400)              | 0.7% (3/400)               | 0.83                  |
| Columnar                                                          | 1.2% (5/400)              | 1.5% (6/400)               | 0.75                  |
| Compliance (%)                                                    | 89% (81–96%)              | 88% (77–99%)               | 0.91                  |
| <i>Peak flow (PEF)</i>                                            |                           |                            |                       |
| AM                                                                | 389 (320–457)             | 410 (308–513)              | 0.35                  |
| PM                                                                | 399 (333–465)             | 422 (323–520)              | 0.37                  |
| <i>Albuterol use:</i>                                             |                           |                            |                       |
| Day (puffs)                                                       | 0.48 (0.21–0.76)          | 0.29 (0.1–0.45)            | 0.22                  |
| Night (puffs)                                                     | 0.79 (0.18–1.39)          | 1.03 (0.35–1.71)           | 0.24                  |
| Methacholine PC <sub>20</sub>                                     | 0.8 mg/ml (95%CI 0.1–1.4) | 0.5 mg/ml (95%CI 0.0–0.9), | 0.43                  |
| <i>Symptoms score</i>                                             |                           |                            |                       |
| Early morning tightness                                           | 0.57 (0.34–0.81)          | 0.49 (0.23–0.74)           | 0.33                  |
| Asthma last night                                                 | 0.16 (0.03–0.28)          | 0.19 (0.00–0.38)           | 0.46                  |
| Asthma during the day                                             | 0.55 (0.32–0.78)          | 0.63 (0.39–0.87)           | 0.30                  |

The following variables were measured and their mean and 95% confidence intervals are displayed. LAR = late allergen response, which is expressed as the change in spirometry over 3–8 h of the study, or percentage fall in FEV<sub>1</sub> per hour. PC<sub>20</sub> allergen, baseline FEV<sub>1</sub>, compliance, and peak flow were analyzed by paired  $t$  test, exhaled NO by ANOVA and percentage differential counts, and albuterol usage and symptoms scores analyzed by Wilcoxon signed-rank test. Values are means with 95% confidence intervals, unless stated otherwise.

<sup>a</sup> AU = allergen units.

\*  $P < 0.05$ .

1.4) versus 0.5 mg/ml (95%CI 0.0–0.9), but this did not achieve significance ( $P = 0.43$ ,  $n = 9$ ). There were no significant differences in asthma symptoms, baseline FEV<sub>1</sub>, PEF, or  $\beta_2$ -agonist use between the two phases.

#### *Ex vivo experiments*

Secretion from the PBMC with and without stimulation by 100 ng/ml LPS demonstrated that baseline (unstimulated) secretion was reduced from 41.8 to 8.2 pg/ml TNF $\alpha$ , and 6 h after LPS from 106.2 to 61.1 pg/ml TNF $\alpha$ . A further reduction was seen if the same cells were incubated in 50% subject serum while taking marimastat (baseline 7.3 and LPS 6 h 33.2 pg/ml TNF $\alpha$ ).

#### **Discussion**

This study suggests that bronchoprotection against allergen may be afforded by a broad spectrum MMP inhibitor such as marimastat, perhaps either via inhibition of TNF $\alpha$  release, or by reducing an alternative effect of MMPs in the airway or a combination of both mechanisms. Increased levels of TNF $\alpha$  and MMP-9 have been implicated in inflammatory cellular migration, airway remodeling, and hyper-responsiveness. Allergen-induced airway inflammation is also associated with elevated levels of eNO, and while the marimastat had no significant effect on reducing exhaled NO levels, during the marimastat phase these subjects inhaled more allergen to achieve a PC<sub>20</sub>, which may explain the higher eNO levels. Alternately, it may be that a MMP inhibitor does not affect NO generation, or, that such an inhibitor affects pathways other than those relating to MMPs and TNF $\alpha$ .

While we achieved the primary endpoint, which was a significant protection against allergen as measured by allergen PC<sub>20</sub>, it is possible that a larger number of subjects would have allowed the demonstration of a significant reduction in airway inflammatory cells, as the fall in the proportion of these cells failed to achieve significance. Sample size calculations were not possible until a pilot study such as this had been performed. Based on the cell data from this study, sample size estimation suggests that 64 subjects completing the study would allow significance to be achieved ( $\alpha = 0.05$ ,  $\beta = 0.8$ ). It is also likely that a larger dose of marimastat might achieve a larger effect, but we wished to err on the side of safety, given that it is a drug with a number of side effects, and these were volunteers who were well and had very mild asthma. Likewise, the reduction in sensitivity to allergen (PC<sub>20</sub>) was modest. There were no data on which we could base our initial sample size calculations, and this pilot study would suggest that a larger dose of marimastat, or a more specific inhibitor of TACE, may be required to have a greater effect. It would have been informative to study the effect of marimastat on an ex vivo sample of blood to ascertain whether adequate levels of this

drug were achieved in terms of an IC<sub>50</sub> for MMPs, especially TACE. To this end, and in view of the modest effect upon PC<sub>20</sub>, a post hoc study of the systemic effect of the marimastat was carried out using marimastat 5 mg twice daily. Secretion from the PBMC with and without stimulation by 100 ng/ml lipopolysaccharide (LPS) demonstrated that baseline (unstimulated) secretion was reduced from 41.8 to 8.2 pg/ml TNF $\alpha$ , and 6 h after LPS from 106.2 to 61.1 pg/ml TNF $\alpha$ . A further reduction was seen if the same cells were incubated in 50% subject serum taken while on marimastat (7.3 and 33.2 pg/ml TNF $\alpha$ , respectively). This suggests that the dose used was appropriate for inhibition of TNF $\alpha$ , but may suggest that the effect of inhibiting this cytokine is not large in allergic asthma.

In addition, it may have been useful to study the effect of this drug upon the skin-prick test responses and to correlate this effect with the bronchial challenge test responses to allergen. In terms of the dose chosen, our concern was not to provoke a side effect in these subjects and none reported the tenosynovitis that has been seen at higher doses. Interestingly, one study of colorectal cancer showed the greatest survival benefit in those who developed musculoskeletal side effects, suggesting that both beneficial and side effects are dose related (King et al., 2003). We may have therefore seen a greater effect if a larger dose was given. The two subjects who dropped out with respiratory infections were both being studied in the winter months, one in the placebo phase and one in the marimastat phase. Other trials in neoplasia have not shown a significant increase in the incidence of infection (Anonymous, 2003).

Airway responses to inhaled allergen are also calculated as early and late allergen responses (EAR and LAR) measured by the area under the percent fall in FEV<sub>1</sub> time response curve for EAR (AUC<sub>0–3 h</sub>) and the LAR (AUC<sub>3–7 h</sub>) (Sterk et al., 1993). In our study, the primary outcome was allergen PC<sub>20</sub> and subjects therefore inhaled varying concentrations of allergen to achieve a PC<sub>20</sub> and had albuterol reversal of their initial bronchoconstriction, making comparisons of changes in FEV<sub>1</sub> time response curve between placebo and marimastat difficult. The significant reduction in FEV<sub>1</sub> during the late phase after marimastat treatment could be attributed to the greater cumulative dose of allergen inhaled by the subjects. Likewise, the study design using allergen PC<sub>20</sub> as an endpoint means that the changes in amount of allergen delivered in the challenge itself could influence the methacholine PC<sub>20</sub>, sputum differential cell count, and the eNO, performed on the same visit. If the same dose of allergen had been given on each visit, a PC<sub>20</sub> could not have been derived, but then these other variables would be more comparable. Albuterol rescue from the allergen PC<sub>20</sub> could also influence methacholine PC<sub>20</sub>.

Significant inhibition of airway reactivity is a feature of other TNF $\alpha$  inhibitors such as pentoxifylline, which has shown a reduction in reactivity in an improved murine model of asthma, also in association with a modest change in airway inflammation in the form of eosinophil recruit-

ment and epithelial thickening (Kumar et al., 2003). TNF $\alpha$  administration can cause an increase in both airway reactivity and inflammatory cells in both normal and asthmatic subjects (Heywood and Thomas, 2002; Thomas et al., 1995), and since it has been implicated in asthma, inhibition of these effects would be expected to reduce bronchial sensitivity to inhaled provocants, as has been demonstrated in this study.

There are data emerging to suggest that MMPs do play a role in the asthmatic inflammatory response: one study found that MMP-9, its inhibitor TIMP-1, and MMP-2 were significantly higher in the sputa of asthmatic subjects in both an exacerbation and during the recovery period when compared to control subjects (Kelly et al., 2000; Lee et al., 2001; Suzuki et al., 2001). Marimastat has an IC<sub>50</sub> of approximately 3 nM for MMP-9 (gelatinase B), and a similar range for MMP-1, -2, -7, -8, -12, and -13 as well (data on file, British Biotech). This value is achieved in man by the dose that was used in this study, and therefore other anti-inflammatory effects mediated by marimastat-induced MMP inhibition may be contributing to the effect seen in these allergic asthmatic subjects.

In summary, this study demonstrates that treatment with a broad spectrum matrix metalloproteinase inhibitor may have a potential role in reducing airway inflammation, as we observed a significant reduction in bronchial hyper-responsiveness to inhaled allergen in asthmatic subjects. Our findings support the view that inflammatory mediators may have the potential to be modified by MMP inhibitors and offer further tentative support for the concept that TNF $\alpha$  plays a role in airway inflammation and remodeling as a possible pathway that can be targeted for reducing allergen-induced airway hyper-responsiveness in asthma.

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