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Toxicology and Applied Pharmacology 205 (2005) 126–132

Toxicology and Applied Pharmacology

www.elsevier.com/locate/ytaap

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

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Received 15 April 2004; accepted 12 October 2004
Available online 15 December 2004

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 (TNFα) 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 β_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_{20} 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_1, asthma symptoms, or albuterol usage.

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

Keywords: TNFα; Asthma; Allergen; Marimastat

Introduction

Tumor necrosis factor alpha (TNFα) 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 TNFα increases airway responsiveness and neutrophil infiltration, in addition, TNFα is increased after allergen inhalation (Keatings et al., 1997; Thomas et al., 1995).

TNFα is released from a cell membrane-anchored precursor by proteolytic cleavage by a TNFα converting enzyme (TACE) or ADAM-17 (ADAM: proteins containing a disintegrin and metalloproteinase domain). In addition, TNFα 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). TNFα 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 TNFα production in vivo via inhibition of MMP activity and hence TNFα release. It was postulated that this would reduce airway inflammatory responses and bronchial hyper-responsiveness that are commonly associated with elevated TNFα production after an allergen challenge.

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doi:10.1016/j.taap.2004.10.005

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 TNFα 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 ± 12) years) were recruited, and eligibility criteria included a forced expiratory volume in 1 s (FEV_1) 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_1) at a provocative concentration (PC_{20}) 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.

Table 1
Baseline characteristics of study subjects

Subject	Sex	Age (years)	Baseline FEV_1% predicted	MCh[a] PC_{20} mg/ml	Predicted[b] *Der p* PC_{20} 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[c]	M	23	72.8	0.9	1.89
11[c]	M	19	80.3	2.5	15.21
12[c]	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.
[a] Methacholine challenge.
[b] Allergen Units (30,000 AU/ml).
[c] Withdrawn from study.

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_1 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 β_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_{20}. Predicted allergen PC_{20} 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_1 measured at 10-min intervals after each inhalation. The challenge was ceased when FEV_1 had fallen by >20% and the concentration of *Der p* in allergen units (AU)/ml that gave a 20% fall in FEV_1 (PC_{20}) was then calculated by interpolation.

Subjects received 200 μg albuterol and FEV_1 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%