Attenuation of early and late phase allergen-induced bronchoconstriction in asthmatic subjects by a 5-lipoxygenase activating protein antagonist, BAYx 1005

A L Hamilton, R M Watson, G Wyile, P M O'Byrne

Abstract

Background **– The cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) have been implicated in the pathogenesis of allergen-induced airway responses. The effects of pretreatment with BAYx 1005, an inhibitor of leukotriene biosynthesis via antagonism of 5-lipoxygenase activating protein, on allergen-induced early and late asthmatic responses has been evaluated.**

Methods **– Eight atopic subjects with mild asthma participated in a two period, double blind, placebo controlled, crossover trial. Subjects were selected on the basis of a forced expiratory volume in one second (FEV_1) of >70% predicted, a methacholine provocative concentration causing a 20% fall in FEV_1 (PC_{20}) of <32 mg/ml, a documented allergen-induced early response (EAR, >15% fall in FEV_1 0–1 hour after allergen inhalation) and late response (LAR, >15% fall in FEV_1 3–7 hours after allergen inhalation), and allergen-induced airway hyperresponsiveness (at least a doubling dose reduction in the methacholine PC_{20} 30 hours after allergen inhalation). During the treatment periods subjects received BAYx 1005 (500 mg twice daily) or placebo for 3.5 days; treatment periods were separated by at least two weeks. On the third day of treatment, two hours after administration of medication, subjects performed an allergen inhalation challenge and FEV_1 was measured for seven hours.**

Results **– Treatment with BAYx 1005 attenuated the magnitude of both the allergen-induced early and late asthmatic responses. The mean (SE) maximal fall in FEV_1 during the EAR was 26.6 (3.3)% during placebo treatment and 11.4 (3.3)% during treatment with BAYx 1005 (mean difference 15.2 (95% confidence interval (CI) 9.4 to 21.00)) with a mean protection afforded by BAYx 1005 of 57.1%. The mean (SE) maximal fall in FEV_1 during the LAR was 19.8 (5.7)% during placebo treatment and 10.7 (4.4)% during BAYx 1005 treatment (mean difference 9.2 (95% CI 1.4 to 17.0)) with a mean protection afforded by BAYx 1005 of 46.0%. The area under the time response curve (AUC_{0-3}) was also reduced after treatment with BAYx 1005 compared with placebo by 86.5%.h (mean difference 26.3 (95% CI 17.1 to 38.5)) and the AUC_{3-7} by 59.6%.h (mean difference 26.9 (95% CI −3.8 to 57.6)).**

Conclusions **– These results show that antagonism of 5-lipoxygenase activating protein can attenuate allergen-induced bronchoconstrictor responses and support an important role for the cysteinyl leukotrienes in mediating these asthmatic responses.**

(*Thorax* 1997;**52**:348–354)

Keywords: asthma, BAYx 1005, leukotriene synthesis inhibition, allergens.

The Asthma Research Group, Department of Medicine, McMaster University, Hamilton, Ontario Canada L8N 3Z5
A L Hamilton
R M Watson
P M O'Byrne

Miles Canada Inc, Ontario, Canada
G Wyile

Correspondence to:
Dr P M O'Byrne.

Received 9 July 1996
Returned to authors 27 September 1996
Revised version received 12 November 1996
Accepted for publication 28 November 1996

Inhalation of environmental allergens is an important cause of exacerbations in asthmatic patients. In the laboratory inhalation of allergen by sensitised subjects causes an early asthmatic response (EAR) within 15 minutes of inhalation, manifested by airway narrowing which usually resolves within 2–3 hours. In more than 50% of adult asthmatic subjects a more prolonged late asthmatic response (LAR) also develops, beginning 2–4 hours after inhalation.[1] The late asthmatic response is often accompanied by airway hyperresponsiveness to bronchoconstrictor stimuli such as histamine or methacholine[2] which can last for days or weeks following allergen exposure.[3] Late asthmatic responses and airway hyperresponsiveness are associated with the influx of inflammatory cells such as eosinophils[4] into the airways.

Considerable evidence is now available to support a role for cysteinyl leukotrienes in the pathogenesis of asthma. The cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) contract airway smooth muscle in vitro[5 6] and inhalation of cysteinyl leukotrienes causes airway narrowing in both normal and asthmatic subjects in vivo.[7–9] Cysteinyl leukotrienes are released from human lungs after allergen challenge in vitro,[10] while increases in cysteinyl leukotrienes in bronchoalveolar lavage (BAL) fluid[11] and increases in urinary LTE_4[12 13] have been observed after allergen challenge in vivo. Cysteinyl leukotrienes also increase microvascular permeability[14] and stimulate secretion of mucus,[15] suggesting a possible involvement in the inflammatory process associated with the LAR. In recent studies pretreatment with specific cysteinyl leukotriene-1 receptor antagonists such as ICI 204 219[16] and MK-571,[17] and leukotriene biosynthesis inhibitors such

Table 1 Subject characteristics

Subject no.	*Age (years)*	*Sex*	*Height (cm)*	*Weight (kg)*	*FEV_1 (l)*	*FEV_1 (%pred)*	*PC_{20}† (mg/ml)*	*Allergen*	*EAR‡ (%fall)*	*LAR‡ (%fall)*	*Treatment sequence*
1	23	M	185	79	4.33	92.1	3.65	Cat dander	25.6	15.4	B/P
2	20	M	172	77	3.67	84.8	3.73	Ragweed	46.9	18.8	B/P
3	41	F	165	61	3.01	81.1	2.68	*D farinae*	25.9	27.6	B/P
4	21	M	167	63	4.78	117.0	18.98	Ragweed	18.4	29.9	P/B
5	26	M	173	69	3.56	85.6	17.97	*D farinae*	19.0	22.2	P/B
6	23	M	185	86	4.33	91.2	1.93	*D farinae*	32.9	25.9	P/B
7	20	M	167	61	3.17	76.6	0.8	Ragweed	24.7	16.9	P/B
8	22	M	180	62	4.60	101.1	2.43	Ragweed	21.0	36.3	P/B
Mean	24.5		174.3	69.8	3.93	91.2	3.83*		26.8	24.1	
(SE)	(2.5)		(2.9)	(3.4)	(0.24)	(4.2)	(1.47)		(3.3)	(2.5)	

EAR = early asthmatic response; LAR = late asthmatic response; FEV_1 = forced expiratory volume in one second; PC_2 = concentration provoking fall in FEV_1 of 20%; B = BAYx 1005; P = placebo.
* Geometric mean (%SE).
† During screening (period 1, day 2).
‡ During screening (period 1, day 3).

as MK-886[18] and MK-0591,[19] have partially attenuated both the EAR and LAR, providing further direct evidence for a role of cysteinyl leukotrienes in the development of both these asthmatic responses.

BAYx 1005 is an indirect leukotriene biosynthesis inhibitor which acts by binding to 5-lipoxygenase activating protein (FLAP), an 18 kD leucocyte membrane protein necessary for the translocation and activation of 5-lipoxygenase. 5-Lipoxygenase is a pivotal enzyme in the leukotriene biosynthetic pathway which binds to FLAP in the nuclear membrane to make a stable complex and subsequently converts arachidonic acid to 5-HPETE and then to LTA_4. LTA_4 is further converted to either LTB_4 or to the cysteinyl leukotrienes LTC_4, LTD_4 and LTE_4. BAYx 1005 antagonises the activity of FLAP, preventing the activation of 5-lipoxygenase and thereby inhibiting leukotriene synthesis.[20]

The purpose of the present study was to evaluate the effects of BAYx 1005 on the allergen-induced EAR and LAR and subsequent increase in airway responsiveness to methacholine in atopic mildly asthmatic subjects.

Methods

SUBJECTS

Nine subjects (seven men) were entered into the study; however, one subject had not demonstrated an allergen-induced LAR during screening, which was a specific criterion for inclusion into the study, and was excluded. The remaining eight subjects (seven men) were included in the statistical analysis. The study was approved by the hospital ethics committees and each subject gave written informed consent before beginning the study. All subjects were studied in the asthma research laboratory at McMaster University Medical Centre. Relevant characteristics of the subjects are shown in table 1. All subjects had a history of mild stable asthma and documentation of asthma exacerbations induced by environmental allergen(s). Subjects were only using inhaled β_2 agonists to treat their asthma on an intermittent basis. Other than a clinical diagnosis of asthma, all subjects were healthy based on medical history, physical examination, electrocardiography, chest radiography, and laboratory screening for haematology, blood chemistry and urinalysis. With the exception of house dust mite, subjects were not currently exposed to allergens to which they were sensitised. Subjects had had no exacerbations of asthma and no respiratory infections for at least four weeks before the start of the investigation. Baseline forced expiratory volume in one second (FEV_1) was >75% predicted normal[21] in all subjects on all study days. All subjects were lifetime non-smokers. Women of child-bearing potential were excluded from participation in the study.

STUDY DESIGN

The study was performed in a double blind, placebo controlled manner with a crossover design. The study was divided into three periods: a screening period (period l) and two treatment periods (periods 2 and 3). On all study days subjects came to the laboratory having refrained from use of inhaled β_2 agonists for at least six hours and had not ingested caffeine-containing products on the morning of each visit. Study periods were separated by at least two weeks.

Screening (period 1)

On day 1 of period 1 spirometric tests were performed to ensure subjects had an FEV_1 of >70% predicted. An allergen skin prick test was performed to determine atopic status, followed by a skin prick titration with doubling dilutions of the allergen producing the greatest weal on the skin test. The allergen end point from the titration was subsequently used to calculate the starting concentration for the allergen inhalation challenge.

On day 2 a diluent control inhalation challenge was performed and FEV_1 was followed for seven hours after the challenge. At the completion of the diluent inhalation challenge a methacholine inhalation challenge was performed to determine the concentration of methacholine which caused fall in FEV_1 of 20% (PC_{20}). The end point of the allergen skin prick titration and the methacholine PC_{20} were used to estimate the PC_{20} of the inhaled allergen extract using the formula described by Cockcroft *et al.*[22]

Day 3 took place at least 24 hours after day 2. An allergen inhalation challenge was performed to document the presence of an early and late asthmatic response to the inhaled allergen, the starting concentration of allergen extract for inhalation was two doubling concentrations below the estimated allergen PC_{20}. On day 4 a methacholine inhalation challenge was performed, 30 hours after the allergen inhalation challenge, to document changes in airway responsiveness following allergen inhalation.

The following specific criteria were used for entry into the treatment period of the study: (1) a methacholine PC_{20} of <32 mg/ml on day 2 of period 1, (2) a documented allergen-induced EAR (defined as a fall in FEV_1 of >15% 0–3 hours after allergen inhalation) and allergen-induced LAR (defined as a fall in FEV_1 of >15% 3–7 hours after allergen inhalation), (3) allergen-induced airway hyperresponsiveness at 30 hours after allergen inhalation (defined as >1 doubling dose decrease in methacholine PC_{20} compared with the methacholine PC_{20} on day 2 of period 1).

Treatment periods (periods 2 and 3)

Subjects completed two treatment periods, one with BAYx 1005 and one with matching placebo. Subjects were entered into the treatment sequence according to a randomised schedule. Each period consisted of five consecutive days. On day 1 a methacholine inhalation challenge was performed; since the airway response to inhaled allergen is, in part, determined by the level of airway responsiveness, each treatment period was started only when the methacholine PC_{20} had returned to within a single doubling concentration of the value determined at day 2 of period 1. Subjects began administration of medication on the morning of the following day (day 2). BAYx 1005 (2×250 mg) or matching placebo were administered orally twice daily (08.00 and 20.00 hours) on days 2, 3, and 4 and on the morning of day 5. On day 3 a methacholine inhalation challenge was performed. On day 4 an allergen inhalation challenge was performed two hours after administration of the morning dose of medication. On day 5 a methacholine challenge was performed 30 hours after allergen inhalation.

CHALLENGE PROCEDURES

Methacholine inhalation challenge

Methacholine inhalation challenges were performed according to the method of Cockcroft *et al.*[23] Subjects inhaled saline followed by increasing doubling concentrations of methacholine chloride using a Wright nebuliser (output 0.13–0.15 ml/min; mass median aerodynamic diameter of particles = 1.3 μm). The nose was clipped and aerosols were inhaled through a mouthpiece during tidal breathing for two minutes. FEV_1 was measured using a water sealed spirometer (Warren E Collins Inc). The test was continued until a 20% fall in FEV_1 from the post-saline baseline value was obtained. The PC_{20} was interpolated from individual dose-response curves drawn on a semilogarithmic non-cumulative scale. Solutions of methacholine chloride were stored at 4°C and administered at room temperature.

Allergen and diluent inhalation challenges

Allergen inhalation challenges were performed as previously described[1] using a Wright nebuliser operated by oxygen at 50 psi and at a flow rate that gave an output of 0.13 ml/min. FEV_1 was measured using a water sealed spirometer, with triplicate FEV_1 measurements at baseline and single FEV_1 measurements after allergen inhalation; volumes were recorded at body temperature, atmospheric pressure, saturated with water vapour. For the screening allergen challenge in period 1 doubling concentrations of allergen were inhaled by tidal breathing (nose clipped) for two minutes, with FEV_1 measured 10 minutes after each inhalation; inhalations were stopped when the FEV_1 had fallen by at least 15% from baseline. FEV_1 was subsequently measured at 20, 30, 45, 60, 90 and 120 minutes and at hourly intervals up to seven hours after allergen inhalation. During the treatment periods (periods 2 and 3) only the three highest concentrations of allergen used in period 1 were inhaled. House dust extracts were obtained from Miles/Hollister-Stier, Mississagua, Ontario, and ragweed and cat dander extracts were obtained from Dr Jerry Dolovich, Hamilton, Ontario. Allergen extracts from the same batch were used during the screening and both treatment periods for each subject. They were stored at −70°C and diluted in phosphate buffered saline with 1.5% benzyl alcohol for skin tests and allergen inhalation on the day of use. The diluent inhalation challenge was performed in the same manner as the allergen inhalation challenge, with subjects inhaling diluent (three inhalations of two minutes) instead of allergen.

MEASUREMENT OF PLASMA LEVELS OF BAYx 1005

On day 4 of periods 2 and 3 (allergen inhalation challenge days) blood samples were obtained prior to administration of medication and at two, four, and six hours after administration for measurement of plasma levels of BAYx 1005.

ANALYSIS OF DATA

Airways responses to inhaled allergen were expressed as the percentage fall in FEV_1 from the pre-allergen baseline value and plotted against time. In addition, for each subject the maximal percentage decrease in FEV_1 from baseline during the EAR and LAR was recorded, and the trapezoidal area under the curve of the percentage change in FEV_1 versus time for the EAR (AUC_{0-3}) and the LAR (AUC_{3-7}) was calculated.

The method of Hills and Armitage[24] for analysis of a two period crossover study was used to compare (1) the maximal percentage decrease in FEV_1 from baseline during the EAR, (2) the maximal percentage decrease in FEV_1

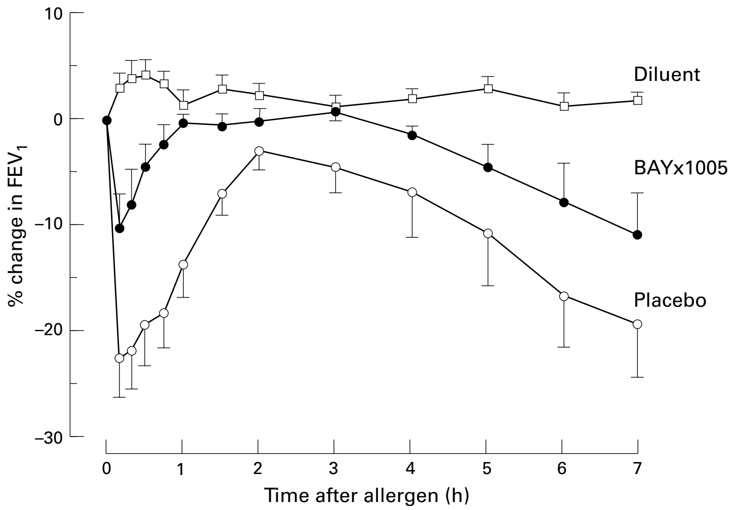

Figure 1 Mean (SE) percentage change in FEV_1 from baseline during the early and late asthmatic response to allergen inhalation after pretreatment with BAYx 1005 and placebo and after inhaled diluent.

from baseline during the LAR, (3) $AUC_{0–3}$, and (4) $AUC_{3–7}$ between the two treatment periods, BAYx 1005 and placebo (n = 8). Summary statistics are expressed as arithmetic mean and standard error of the mean.

All analyses of methacholine PC_{20} were performed on log transformed values with summary statistics expressed as geometric mean and percentage standard error (%SE). The effect of BAYx 1005 and placebo on allergen-induced increases in airway responsiveness was assessed by comparing the difference (log PC_{20} (30 hours after allergen inhalation) − log PC_{20} (24 hours before allergen inhalation)) using the method of Hills and Armitage[24] for analysis of a two period crossover study (n = 6); a negative difference signified an increase in airway responsiveness. Two subjects had to be excluded from this analysis, one who did not take the final two doses of medication during both treatment periods and a second who had a protocol violation during the screening period and did not demonstrate allergen-induced airway hyperresponsiveness. Probability values of <0.05, two tailed, were considered statistically significant. In all analyses tests for period and carryover effects were performed and no significant effects were observed.

Table 2 Mean (SE) early and late airway responses and methacholine responsiveness following allergen inhalation during treatment with BAYx 1005 and placebo

	Placebo	*BAYx 1005*	*% inhibition*	*p value*
Baseline FEV_1	3.63 (0.24)	3.81 (0.22)		0.17
EAR (0–3 hours)				
Maximum % fall FEV_1	26.6 (3.3)	11.4 (3.3)	57.1	<0.001
AUC FEV_1	30.4 (5.3)	4.1 (3.2)	86.5	<0.001
LAR (3–7 hours)				
Maximum % fall FEV_1	19.8 (5.7)	10.7 (4.4)	46.0	0.03
AUC FEV_1	45.1 (18.5)	18.2 (8.7)	59.6	0.075
log difference PC_{20}	−0.30 (0.14)	−0.12 (0.10)		0.35

EAR = early asthmatic response; LAR = late asthmatic response; FEV_1 = forced expiratory volume in one second; AUC = area under curve; log difference PC_{20} = (log PC_{20} after allergen – log PC_{20} before allergen).

Results

There was no significant difference in baseline FEV_1 before allergen between treatment periods, the FEV_1 being 3.81 (0.22) l during BAYx 1005 treatment and 3.63 (0.24) l during placebo treatment. The mean percentage fall in FEV_1 from baseline up to seven hours after allergen inhalation during the BAYx 1005 and placebo treatment periods is shown in fig 1. The maximum percentage change in FEV_1 from baseline was significantly reduced after treatment with BAYx 1005, both during the EAR and the LAR (table 2). The maximal percentage fall in FEV_1 during the EAR was 26.6 (3.3)% during placebo treatment and 11.4 (3.3)% during treatment with BAYx 1005 (mean difference 15.2 (95% confidence interval (CI) 9.4 to 21.0); p<0.001). The maximal percentage fall in FEV_1 during the LAR was 19.8 (5.7)% during placebo treatment and 10.7 (4.4)% during treatment with BAYx 1005 (mean difference 9.2 (95% CI 1.4 to 17.0); p = 0.03). The $AUC_{0–3}$ was also significantly reduced after treatment with BAYx 1005 compared with placebo (table 2).

The baseline methacholine PC_{20} was not significantly different between treatment periods (mean (%SE) 2.80 (1.62) mg/ml during placebo treatment and 3.42 (1.64) mg/ml during BAYx 1005 treatment) or before allergen inhalation (2.67 (1.60) mg/ml during placebo treatment and 3.74 (1.61) mg/ml during BAYx 1005 treatment). Furthermore, there was no significant difference in methacholine PC_{20} between treatment with either BAYx 1005 or placebo. Allergen inhalation did not cause a significant change in the methacholine PC_{20} during treatment with BAYx 1005 (PC_{20} 3.74 (1.61) mg/ml before and 2.87 (1.60) mg/ml 30 hours after allergen). However, despite a significant allergen-induced decrease in methacholine PC_{20} of 1.7 doubling dilutions during the screening period, allergen inhalation did not cause a significant change in the methacholine PC_{20} during treatment with placebo (methacholine PC_{20} 2.67 (1.60) mg/ml 24 hours before and 1.35 (1.56) mg/ml 30 hours after allergen). Thus, the change in logPC_{20} from 24 hours before allergen to 30 hours after allergen was not significantly different between BAYx 1005 and placebo treatments (table 2).

On the day of the allergen challenge, after two days of treatment with BAYx 1005, the plasma concentrations of BAYx 1005 were 6.68 (1.41), 9.36 (1.14), 9.85 (1.13), and 9.98 (2.59) µg/ml before and two, four, and six hours after the morning dose of BAYx 1005. BAYx 1005 was well tolerated in all subjects. No clinically significant changes in physical examination, serum chemistry, haematological parameters, urine parameters, or electrocardiography were observed.

Discussion

This study has shown that BAYx 1005, a leukotriene biosynthesis inhibitor that acts through antagonism of 5-lipoxygenase activating protein, given for three days produced a partial reduction in both the EAR and LAR following allergen inhalation challenge in patients with mild asthma. These results add support to the hypothesis that cysteinyl leukotrienes are important mediators, not only during the early response but also during the late response following allergen inhalation.

There is now considerable evidence available to suggest that leukotrienes may have a role in the pathogenesis of bronchial asthma. Leukotrienes are produced by both constitutive cells (mast cells, alveolar macrophages) and by infiltrating cells (eosinophils) within the lungs.[10] Urinary levels of LTE_4[12 13] and levels of cysteinyl leukotrienes in BAL fluid[11] are increased following allergen challenge. Cysteinyl leukotrienes are potent contractile agonists for bronchial smooth muscle in vitro[5 6] and aerosolised cysteinyl leukotrienes induce potent bronchoconstriction in both normal and asthmatic subjects in vivo.[7–9] Inhalation of cysteinyl leukotrienes also induces eosinophilia in the airways[25 26] and increases airway responsiveness to histamine in asthmatic subjects.[27] They are potent stimulants of mucus glycoproteins from human airways in vitro,[15] enhance secretion of mucus in canine trachea in vivo,[28] and cause vasoconstriction and increased vascular permeability in the airways of guinea pigs.[14] In addition, LTB_4 induces neutrophil chemotaxis and aggregation and neutrophil-endothelial cell adhesion, enhances microvascular permeability, and induces neutrophil degranulation and lysosomal enzyme release.[29] Thus, as well as being suitable for mediation of the acute bronchoconstrictor response, there is evidence to suggest a role for leukotrienes in the inflammatory process involved in the development of the LAR.

First generation cysteinyl leukotriene receptor antagonists (LY171 883, L649 923, L648 051) showed modest efficacy in attenuating the allergen-induced EAR, but had little or no effect on the LAR. Second generation, highly selective and potent cysteinyl leukotriene receptor antagonists (ICI 204 219,[16] MK-571[17]) have been more efficacious, attenuating the EAR by up to 80%, with more modest but significant attenuation of the LAR (up to 50%). The new potent leukotriene biosynthesis inhibitors offer a potential advantage over the cysteinyl leukotriene receptor antagonists by inhibiting the biosynthesis of both cysteinyl leukotrienes and LTB_4. Although Zileuton and ZD2138, selective 5-LO inhibitors, failed to inhibit allergen-induced airway responses,[30 31] the potent FLAP antagonists MK-886 and MK-0591 significantly attenuated both the EAR and the LAR.[18 19]

BAYx 1005 is a potent and selective quinoline derived leukotriene synthesis inhibitor in in vitro systems, including human and rat polymorphonuclear leucocytes.[20 32] In studies with human polymorphonuclear leucocytes BAYx 1005 was found to be 800 times more potent in intact cells than in cell-free systems. The Kd for binding to the high affinity binding site (0.165, μmol/l) was almost identical to the IC_{50} value for inhibition of LTB_4 synthesis (0.22 μmol/1) in calcium ionophore A23187-stimulated leucocytes. In addition, the IC_{50} values for competition of BAYx 1005 by the FLAP antagonist MK-886 corresponded to the IC_{50} values for MK-886 inhibition of LTB_4 synthesis (0.09 μmol/1 and 0.07 μmol/l, respectively), suggesting that BAYx 1005 shares the same working mechanism as MK-886 and may be considered to be a FLAP antagonist.

BAYx 1005 has been shown to block IgE mediated contractions of human airways pretreated with atropine, indomethacin, and chlorpheniramine with a 10 times greater potency than MK-886; it also prevented the increase in LTE_4 levels that was observed during the anti-IgE challenge.[33] BAYx 1005 has also been shown to be effective in preclinical in vivo models of acute inflammation. In an arachidonic acid evoked mouse ear inflammation test BAYx 1005 was both topically (ED_{50} 18 μg/ear) and systemically (ED_{50} 48.7 mg/kg orally) active in inhibiting oedema formation.[34]

We may compare the results of the present study with other studies that have examined the effects of FLAP antagonists on allergen-induced airway responses. With regard to allergen-induced EAR, treatment with BAYx 1005, administered at a dose of 500 mg twice daily for three days, appears to have been more effective than treatment with MK-886 administered in two oral doses of 500 mg and 250 mg one hour before and two hours after allergen inhalation,[18] and comparable to treatment with MK-0591 administered in doses of 250 mg at 24, 12, and 1.5 hours before inhalation of allergen.[19] BAYx 1005, MK-886, and MK-0591 inhibited the maximum percentage fall in FEV_1 during the EAR by 57.1%, 28.7%, and 57.0%, respectively, and the AUC_{0-3} by 86.5%, 58.4%, and 79.1%, respectively.

Treatment with BAYx 1005 was more effective than MK-886 during the LAR (3–7 hours after allergen), attenuating the maximum percentage fall in FEV_1 by 46.0% and the AUC_{3-7} by 59.6% compared with inhibitions of 19.1% in the maximum percentage fall in FEV_1 and 43.6% in the AUC_{3-7} with MK-886. However, the reduced effectiveness of MK-886 during the LAR may be explained partly by the pharmacokinetics of MK-886 which has a short half life of two hours. Subject withdrawals after six hours in the study reported by Diamant and colleagues makes it difficult to compare the effectiveness of BAYx 1005 and MK-0591 during the LAR in terms of maximum percentage fall in FEV_1 and AUC_{3-7}. However, it does appear that MK-0591 and BAYx 1005 were comparably effective with respect to the reduction in the mean percentage fall in FEV_1at each time point from three to six hours; at six hours after allergen inhalation there was a reduction of approximately 50% in the mean percentage fall in FEV_1 with MK-0591 and a 54% reduction in the mean percentage fall in FEV_1 with BAYx 1005.

While the similarities in the extent that BAYx 1005 and MK-0591 inhibited the EAR and the LAR (up to six hours after allergen) point to a similar degree of inhibition of leukotriene biosynthesis, we do not have any data concerning the degree of inhibition of leukotriene synthesis by BAYx 1005 in the present study, since measurements of LTB_4 biosynthesis and urinary LTE_4 were not attempted. Diamant and colleagues[19] found that treatment with MK-0591 blocked LTB_4 biosynthesis stimulated by the calcium ionophore A-23187 in whole blood ex vivo by at least 96% and inhibited excretion of urinary LTE_4 by 87% from pretreatment values for 0–24 hours after allergen challenge. In a recent study Dahlén and colleagues[35] measured urinary levels of LTE_4 after allergen challenge following pretreatment with BAYx 1005 (750 mg, four hours before allergen) and observed a 76% inhibition of excretion of urinary LTE_4 compared with placebo in the first two hours after challenge. The mean plasma concentration of BAYx 1005 was 10.5 mg/l (range 5.3–19.8 mg/l) at four hours after treatment which compares with a mean concentration of 9.85 mg/l (range 4.4–14.4 mg/l) at the same time point in our study.

If LTB_4 plays a significant part in the pathophysiology of allergen-induced asthma, then FLAP antagonists should provide an increased protection compared with cysteinyl leukotriene receptor antagonists which block the actions of cysteinyl leukotrienes but do not impinge on the biological activity of LTB_4. The lack of any marked improvement in protection with BAYx 1005 and MK-0591 compared with potent cysteinyl leukotriene receptor antagonists such as ICI 204219 and MK-571 casts doubt on the role of LTB_4 in allergen-induced asthma. Pretreatment with ICI 204 219 (2×20 mg) two hours before allergen challenge resulted in a reduction of 80.6% in the maximum percentage fall in FEV_1 during the EAR and of 54.5% at the six hour time point during the LAR,[16] while administration of 450 mg MK-571 intravenously resulted in reductions in the maximum percentage fall in FEV_1 of 62.4% and 49.6% during the EAR and LAR (3–10 hours after the challenge), respectively.[17]

The mechanism by which BAYx 1005 attenuates the EAR is probably through modulation of the cysteinyl leukotriene constrictor effects on bronchial smooth muscle. While a similar mechanism may also be invoked to explain attenuation of the LAR, the cited pro-inflammatory properties of leukotrienes may suggest a possible alternative mechanism by which BAYx 1005 acts via modulation of leukotriene mediated influx of inflammatory cells into the airways. Further studies are needed to examine the influx of inflammatory cells into the airways during treatment with leukotriene biosynthesis inhibitors and cysteinyl leukotriene receptor antagonists.

Our attempts to examine the effect of BAYx 1005 on allergen-induced airway hyperresponsiveness following allergen inhalation were prevented by the lack of a significant decrease in the methacholine PC_{20} following placebo treatment. Results from other studies that have examined the effects of cysteinyl leukotriene antagonists and leukotriene biosynthesis inhibitors on allergen-induced airway hyperresponsiveness have not shown agreement. Taylor and colleagues[16] observed a significant attenuation of allergen-induced airway hyperresponsiveness following pretreatment with the cysteinyl leukotriene receptor antagonist ICI 204 219, but Friedman and colleagues[18] and Diamant *et al*[19] did not observe such an effect following treatment with the FLAP antagonists MK-886 and MK-0591. A recent study from our laboratory has calculated that the appropriate sample size to demonstrate a treatment induced attenuation of allergen-induced airway hyperresponsiveness of 50% with a power of 90% is approximately 15 subjects.[36] Studies using larger sample sizes are required to establish conclusively whether these potent biosynthesis inhibitors and cysteinyl leukotriene antagonists are, indeed, effective inhibitors of allergen-induced airway hyperresponsiveness.

In summary, the results of this study have shown that the leukotriene biosynthesis inhibitor BAYx 1005 partially attenuates both allergen-induced EAR and LAR, adding further support to the hypothesis that leukotrienes are important mediators in both the early and late asthmatic responses after allergen inhalation in asthmatic subjects.

This study was supported by the Medical Research Council of Canada and a grant from Miles Canada. P M O'Byrne is a recipient of a Medical Research Council Senior Scientist Award.

1 O'Byrne PM, Dolovich J, Hargreave FE. State of the art: late asthmatic responses. *Am Rev Respir Dis* 1987;**36**: 740–51.
2 Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in nonallergic bronchial reactivity. *Clin Allergy* 1977;7:503–13.
3 Cartier A, Thomson NC, Frith PA, Roberts R, Hargreave FE. Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. *J Allergy Clin Immunol* 1982; **70**:170–7.
4 De Monchy JGR, Kauffman HF, Venge P, Koeter GH, Jansen HAI, Sluiter HJ, *et al*. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reaction. *Am Rev Respir Dis* 1985;**131**:373–6.
5 Dahlen S-E, Hedqvist P, Hammarstrom S, Samuelsson B. Leukotrienes are potent constrictors of human bronchi. *Nature* 1980;**288**:484–6.
6 Hanna CJ, Bach MK, Pare PD, Schellenberg RR. Slow-reacting substances (leukotrienes) contract human airway and pulmonary vascular smooth muscle in vitro. *Nature* 1981;343–4.
7 Barnes NC, Piper PJ, Costello JF. Comparative effects of inhaled leukotriene C4, leukotriene D4, and histamine in normal human subjects. *Thorax* 1984;**39**:500–4.
8 Adelroth E, Morris MM, Hargreave FE, O'Byrne PM. Airway responsiveness to leukotrienes C4 and D4 and to methacholine in patients with asthma and normal controls. *N Engl J Med* 1986;**315**:480–4.
9 Davidson AB, Lee TH, Scanlon PD, Solway J, McFadden ER, Ingram RH, *et al*. Bronchoconstrictor effects of leukotriene E_4 in normal and asthmatic subjects. *Am Rev Respir Dis* 1987;**135**:333–7.
10 Drazen JM, Austen KF. State of the art: leukotrienes and airway responses. *Am Rev Respir Dis* 1987;**136**:985–98.
11 Wenzel SE, Larsen GL, Johnston K, Voelkel NF, Westcott JY. Elevated levels of leukotriene C_4 in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. *Am Rev Respir Dis* 1990;**142**:112–9.
12 Manning PJ, Rokach J, Malo JL, Ethier D, Cartier A, Girard Y, *et al*. Urinary leukotriene E_4 levels during early and late asthmatic responses. *J Allergy Clin Immunol* 1990;**86**: 211–20.
13 Taylor GW, Black P, Turner N, Taylor I, Maltby NH, Fuller RW, *et al*. Urinary leukotriene E_4 after antigen challenge and in acute asthma and allergic rhinitis. *Lancet* 1989;i: 584–7.
14 Woodward DF, Wasserman MA, Weichmann BM. Investigation of leukotriene involvement in the vaso-permeability response associated with guinea-pig tracheal anaphylaxis: comparison with cutaneous anaphylaxis. *Eur J Pharmacol* 1983;**93**:9–19.
15 Marom Z, Shelhamer JH, Bach MK, Morton DR, Kaliner M. Slow-reacting substances, leukotrienes C4 and D4, increase the release of mucus from human airways in vitro. *Am Rev Respir Dis* 1982;**126**:449–51.
16 Taylor IK, O'Shaugnessy KM, Fuller RW, Dollery CT. Effect of cysteinyl-leukotriene receptor antagonist ICI 204 219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. *Lancet* 1991;**337**:690–4.

17 Rasmussen JB, Eriksson L-O, Margolskee DJ, Tagari P, Williams VC, Andersson K-E. Leukotriene D_4 receptor blockade inhibits the immediate and late bronchoconstrictor responses to inhaled antigen in patients with asthma. *J Allergy Clin Immunol* 1992;**90**:l93–201.
18 Friedman BS, Bel EH, Buntinx A, Tanaka W, Han Y-HR, Shingo S, *et al*. Oral leukotriene inhibitor (MK-886) blocks allergen-induced airway responses. *Am Rev Respir Dis* 1993;**147**:839–44.
19 Diamant Z, Timmers MC, van der Veen H, Friedman BS, de Smet M, Depre M, *et al*. The effect of MK-0591, a novel 5-lipoxygenase activating protein inhibitor, on leukotriene biosynthesis and allergen-induced airway responses in asthmatic subjects in vivo. *J Allergy Clin Immunol* 1995;**95**:42–51.
20 Hatzelmann A, Fruchtmann R, Mohrs KH, Raddatz S, Muler-Peddinghaus R. Mode of action of the new selective leukotriene synthesis inhibitor BAYx 1005 {(R)-2-[4-(Quinolin-2-yl methoxy)phenyl]-2-cyclopentyl] acetic acid} and structurally related compounds. *Biochem Pharmacol* 1993;**45**:101–11.
21 Morris JF, Koski A, Johnson LC. Spirometric standards for healthy nonsmoking adults. *Am Rev Respir Dis* 1971;**103**:57–67.
22 Cockcroft DW, Murdock KY, Kirby J, Hargreave FE. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. *Am Rev Respir Dis* 1987;**135**:264–7.
23 Cockcroft DW, Killian DN, Mellon JJA, Hargreave PK. Bronchial reactivity of inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;**7**:235–43.
24 Hills M, Armitage P. The two-period cross-over clinical trial. *Br J Clin Pharmacol* 1979;**8**:7–20.
25 Laitinen LA, Laitinen A, Haahtela T, Vikka V, Spur BW, Lee TH. Leukotriene E_4 and granulocytic infiltration into asthmatic airways. *Lancet* 1993;**341**:989–90.
26 Diamant Z, van Rensen ELJ, Callenbach PMC, Veselic-Charvat MA, van der Veen H, Sterk PJ. Cell differentials in induced sputum after inhaled leukotriene D_4 in subjects with mild asthma. *Am J Respir Crit Care Med* 1996;**153**:A290.
27 Arm JP, Spur BW, Lee TH. The effects of inhaled leukotriene E_4 on the airway responsiveness to histamine in subjects with asthma and normal subjects. *J Allergy Clin Immunol* 1988;**82**:654–60.
28 Johnson HG, McNee ML. Secretagogue responses of leukotriene C_4, D_4: comparison of potency in canine trachea *in vivo*. Prostaglandins 1983,**25**:237–43.
29 Henderson WR. The role of leukotrienes in inflammation. *Ann Intern Med* 1994;**121**:68–97.
30 Hui KP, Taylor IK, Taylor GW, Rubin P, Kesterson J, Barnes NC, *et al*. Effect of a 5-lipoxygenase inhibitor on leukotriene generation and airway responses after allergen challenge in asthmatic patients. *Thorax* 1991;**46**:184–9.
31 Nasser SM, Bell GS, Hawksworth RJ, Spruce KE, MacMillan R, Williams AJ, *et al*. Effect of the 5-lipoxygenase inhibitor ZD2138 on allergen-induced early and late asthmatic responses. *Thorax* 1994;**49**:743–8.
32 Fruchtman R, Mohrs KH, Hatzelmann A, Raddatz S, Fugmann B, Junge B, *et al*. In vitro pharmacology of BAYx 1005, a new inhibitor of leukotriene synthesis. *Agents Actions* 1993;**38**:188–95.
33 Gorenne I, Labat C, Gascard JP, Norel X, Muller-Peddinghaus R, Mohrs KH, *et al*. (R)2-[4-(Quinolin-2-yl-methoxy)phenyl]-2-cyclopentyl] acetic acid (BAYx 1005), a potent leukotriene synthesis inhibitor: effects on anti-IgE challenge in human airways. *J Pharmacol Exp Ther* 1994;**268**:868–72.
34 Muller-Peddinghaus R, Kohlsdorfer C, Theisen-Popp P, Fruchtman R, Perzborn E, Beckermann B, *et al*. BAYx 1005, a new inhibitor of leukotriene synthesis: in vivo inflammation pharmacology and pharmacokinetics. *J Pharmacol Exp Ther 1993;***267**:51–7.
35 Dahlén B, Kumlin M, Ihre E, Zetterstrom O, Dahlén S-E. Inhibition of allergen-induced airway obstruction and leukotriene generation in atopic asthmatic subjects by the leukotriene biosynthesis inhibitor BAYx 1005. *Thorax* 1997;**52**:342–7.
36 Inman MD, Watson R, O'Byrne PM. Reproducibility and utility of methacholine airway responsivenes in studies of allergen-induced asthma. *Am J Respir Crit Care Med* 1996, **153**:A623.