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

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Abstract

Background – The cysteinyl leukotrienes (LTC₄, LTD₄, and LTE₄) 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 atop subjects with mild asthma participated in a two period, double blind, placebo controlled, cross-over trial. Subjects were selected on the basis of a forced expiratory volume in one second (FEV₁) of >70% predicted, a methacholine provocative concentration causing a 20% fall in FEV₁ (PC₂₀) <32 mg/ml, a documented allergen-induced early response (EAR, >15% fall in FEV₁, 0–1 hour after allergen inhalation) and late response (LAR, >15% fall in FEV₁, 3–7 hours after allergen inhalation), and allergen-induced airway hyperresponsiveness (at least a doubling dose reduction in the methacholine PC₂₀, 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 FEV₁ 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 FEV₁ 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 FEV₁ 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 (AUC₀₋₃) 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 AUC₉₋₁ 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.

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

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. The late asthmatic response is often accompanied by airway hyperresponsiveness to bronchoconstrictor stimuli such as histamine or methacholine which can last for days or weeks following allergen exposure. Late asthmatic responses and airway hyperresponsiveness are associated with the influx of inflammatory cells such as eosinophils into the airways.

Considerable evidence is now available to support a role for cysteinyl leukotrienes in the pathogenesis of asthma. The cysteinyl leukotrienes (LTC₄, LTD₄, and LTE₄) contract airway smooth muscle in vitro and inhalation of cysteinyl leukotrienes causes airway narrowing in both normal and asthmatic subjects in vivo. Cysteinyl leukotrienes are released from human lungs after allergen challenge in vitro, while increases in cysteinyl leukotrienes in bronchoalveolar lavage (BAL) fluid and increases in urinary LTE₄ have been observed after allergen challenge in vivo. Cysteinyl leukotrienes also increase microvascular permeability and stimulate secretion of mucus, 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 and MK-571, and leukotriene biosynthesis inhibitors such
as MK-886 and MK-0591 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 kDa 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-HETE and then to LTA4. LTA4 is further converted to either LTE4 or to the cysteinyl leukotrienes LTc4, LTD4, and LTE4. BAYx 1005 antagonises the activity of FLAP, preventing the activation of 5-lipoxygenase and thereby inhibiting leukotriene synthesis.

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 and urinalysis. With the exception of house dust mite, subjects were not currently exposed to allergens to which they were sensitised. Subjects 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 (FEV₁) was >75% predicted normal 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 1) and two treatment periods (periods 2 and 3). On all study days subjects came to the laboratory having refrained from use of inhaled β₂ 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.

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 allergens. Subjects were only using inhaled β₂ 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

Table 1 Subject characteristics

<table>
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<tr>
<th>Subject no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>FEV₁ (l)</th>
<th>FEV₁ (%pred)</th>
<th>PC₂₀ (mg/ml)</th>
<th>Allergen</th>
<th>EAR (%)fall</th>
<th>LAR (%)fall</th>
<th>Treatment sequence</th>
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<td>15.4</td>
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<td>84.8</td>
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<td>81.1</td>
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<td>Cat dander</td>
<td>26.8</td>
<td>24.1</td>
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Note: EAR = early asthmatic response; LAR = late asthmatic response; FEV₁ = forced expiratory volume in one second; PC₂₀ = concentration provoking fall in FEV₁ of 20%; B = BAYx 1005; P = placebo.
* Geometric mean (%SE).
† During screening (period 1, day 2).
‡ During screening (period 1, day 3).
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. 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 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 allergens 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, Mississauga, 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 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}...
Results

There was no significant difference in baseline FEV₁ before allergen between treatment periods, the FEV₁ being 3.81 (0.22) l during BAYx 1005 treatment and 3.63 (0.24) l during placebo treatment. The mean percentage fall in FEV₁ 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 FEV₁ from baseline 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 FEV₁ 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 AUC₀–₃ was also significantly reduced after treatment with BAYx 1005 compared with placebo (table 2).

The baseline methacholine PC₂₀ was not significantly different between 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 PC₂₀ were performed on log transformed values with summary statistics expressed as geometric mean PC₂₀ 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 PC₂₀ (30 hours after allergen inhalation) – log PC₂₀ (24 hours before allergen inhalation)) using the method of Hills and Armitage 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.

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 cysteinyi 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. Inhalation of cysteinyi leukotrienes also induces eosinophilia in the airways and increases airway responsiveness to histamine in asthmatic subjects. They are potent stimulants of mucus glycoproteins from human airways in vitro and aerosolised cysteinyi leukotrienes induce potent bronchoconstriction in both normal and asthmatic subjects in vivo. Inhalation of cysteinyi leukotrienes also induces eosinophilia in the airways and increases airway responsiveness to histamine in asthmatic subjects. 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 cysteinyi 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 cysteinyi leukotriene receptor antagonists (ICI 204 219, MK-571) 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 cysteinyi leukotriene receptor antagonists by inhibiting the biosynthesis of both cysteinyi leukotrienes and LTB4. Although Zileuton and ZD2138, selective 5-LO inhibitors, failed to inhibit allergen-induced airway responses, the potential FLAP antagonists MK-886 and MK-0591 significantly attenuated both the EAR and the LAR.

BAYx 1005 is a potent and selective quinoline-derived leukotriene synthesis inhibitor in vitro systems, including human and rat polymorphonuclear leucocytes. 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 ± 0.02 μmol/l) was almost identical to the IC50 value for inhibition of LTB4 synthesis (0.22 ± 0.07 μmol/l) with calcium ionophore A23187-stimulated leucocytes. In addition, the IC50 values for competition of BAYx 1005 by the FLAP antagonist MK-886 corresponded to the IC50 values for MK-886 inhibition of LTB4 synthesis (0.09 ± 0.02 μmol/l and 0.07 ± 0.01 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 pre-treated with atropine, indomethacin, and chlorpheniramine with a 10 times greater potency than MK-886; it also prevented the increase in LTE4 levels that was observed during the anti-IgE challenge. 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 (ED50 18 μg/ear) and systemically (ED50 48.7 mg/kg orally) active in inhibiting oedema formation.

We may compare the results of the present study 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, and comparable to treatment with MK-0591 administered in doses of 250 mg at 24, 12, and 1.5 hours before inhalation of allergen. BAYx 1005, MK-886, and MK-0591 inhibited the maximum percentage fall in FEV1 during the EAR by 57.1%, 28.7%, and 57.0%, respectively, and the AUC0±3 was 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 FEV1 by 46.0% and the AUC3±7 by 59.6% compared with inhibitions of 19.1% in the maximum percentage fall in FEV1 and 43.6% in the AUC3±7 with MK-886. However, the reduced effect of MK-886 on 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 FEV1 and AUC3±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 FEV1 at 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 FEV1 with MK-0591 and a 54% reduction in the mean percentage fall in FEV1 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 LTB4 biosynthesis and urinary LTE4 were not attempted. Diamant and colleagues10 found that treatment with MK-0591 blocked LTB4 biosynthesis stimulated by the calcium ionophore A-23187 in whole blood ex vivo by at least 96% and inhibited excretion of urinary LTE4 by 87% from pretreatment values for 0–24 hours after allergen challenge. In a recent study Dahlen and colleagues16 measured urinary levels of LTE4 after allergen challenge following pretreatment with BAYx 1005 (750 mg, four hours before allergen) and observed a 76% inhibition of excretion of urinary LTE4 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 LTB4 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 LTB4. 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 LTβ4 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 FEV1 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 FEV1 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 constrictive 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 PC20 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 colleagues11 observed a significant attenuation of allergen-induced airway hyperresponsiveness following pretreatment with the cysteinyl leukotriene receptor antagonist ICI 204 219, but Friedman and colleagues18 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 30% with a power of 90% is approximately 15 subjects.30 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.


