

Inhibition of allergen-induced airway obstruction and leukotriene generation in atopic asthmatic subjects by the leukotriene biosynthesis inhibitor BAYx 1005

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Abstract

Background – Leukotriene receptor antagonists significantly blunt allergen-induced bronchoconstriction in asthmatic subjects. Inhibitors of leukotriene synthesis should theoretically provide similar protection, but conflicting results have been obtained when synthesis inhibitors have been tested in allergen challenge. BAYx 1005, a new inhibitor of leukotriene synthesis, was therefore evaluated in an allergen bronchoprovocation study.

Methods – Ten men with mild allergic asthma and bronchial hyperresponsiveness to histamine were recruited. On two different occasions each subject inhaled a single dose of allergen, previously determined to cause at least a 20% fall in forced expiratory volume in one second (FEV₁) four hours after ingestion of 750 mg BAYx 1005 or placebo in a double blind crossover design. Urinary excretion of leukotriene E₄ was measured before and during the challenges.

Results – The mean (SE) maximal fall in FEV₁ was 7.1 (1.7)% after BAYx 1005 and 21.0 (3.0)% after placebo (p<0.001). The mean difference between treatments was 13.9 (95% CI 7.0 to 20.8) for the maximal fall in FEV₁. All subjects were protected by BAYx 1005, the mean inhibition of the fall in FEV₁ being 70.0 (7.0)%. The mean area under the curve (AUC) for urinary excretion of leukotriene E₄ in the first two hours after the challenge was 1.7 (0.9) after placebo and 0.4 (0.6) after BAYx 1005 (difference = 1.3 (95% CI -0.1 to 2.7); p<0.05).

Conclusions – These results indicate that BAYx 1005 is a potent inhibitor of allergen-provoked leukotriene synthesis in asthmatic subjects and lend further support to the suggestion that leukotrienes are important mediators of allergen-induced bronchoconstriction.

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of leukotrienes. Among such antileukotriene drugs, specific receptor antagonists of the cysteinyl leukotrienes (LTC₄, LTD₄ and LTE₄) have been found to inhibit asthmatic reactions induced by allergens,³⁻⁶ exercise,⁷⁻⁹ and aspirin.^{10,11}

Another class of antileukotriene drugs is represented by compounds which inhibit the biosynthesis of leukotrienes by inhibition of the 5-lipoxygenase or its activation. BAYx 1005 inhibits the production of leukotrienes¹² by antagonism of the 5-lipoxygenase activating protein (FLAP)¹³ which has a pivotal role in the stimulation-evoked synthesis of leukotrienes from arachidonic acid. Consistent with this mode of action, BAYx 1005 has been found to inhibit IgE-dependent leukotriene formation in human lung in vitro, as well as the allergen-induced reactions in isolated human bronchi and animal models of asthma.¹⁴⁻¹⁶

This study evaluates the effect of BAYx 1005 on allergen-induced bronchoconstriction in a group of atopic asthmatic subjects. Furthermore, allergen provocation of asthmatic subjects is associated with release of LTE₄ into the urine¹⁷⁻²⁰ because a significant proportion of cysteinyl leukotrienes formed in the lungs is excreted by this route,^{21,22} so the effect of BAYx 1005 on endogenous formation of leukotrienes was assessed by serial measurements of urinary levels of LTE₄.

Despite the current development of antileukotriene drugs for clinical treatment of asthma, a limited number of studies have been published on the effects of different antileukotriene drugs on allergen challenge. In particular, studies on the influence of leukotriene biosynthesis inhibitors on allergic bronchoconstriction are few and have produced variable results. For example, the 5-lipoxygenase inhibitors Zileuton and ZD 2138 failed to cause significant inhibition of allergen-induced bronchoconstriction^{23,24} whereas two FLAP antagonists, MK-886 and MK-591, were found to inhibit the response.^{25,26}

Methods

PATIENTS

Ten non-smoking atopic men with a history of asthma and with allergy to grass pollen, cat or dog dander participated in the study (table 1). Their asthma was stable and controlled by inhaled β₂ agonists as required. During the study the subjects actively avoided contact with

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Recent experimental and clinical observations suggest that leukotrienes are important mediators of asthma and other inflammatory diseases.^{1,2} Drugs have therefore been developed to inhibit the action or formation

Table 1 Subject characteristics

Patient no.	Age (years)	FEV ₁ (% pred)	Histamine PD ₂₀ (µg)	Allergen	Dose (SQ) ¹
1	46	76	77	Dog	71
2	24	93	29	Grass	710
3	19	106	220	Cat	210
4	31	100	940	Cat	781
5	29	84	10	Dog	7
6	33	88	60	Cat	710
7	28	79	160	Cat	213
8	23	80	13	Dog	71
9	25	87	415	Cat	710
10	25	97	128	Cat	923
Mean	28	89	88 ²		
Range	19–46	76–106	10–940		

¹ Dose used during the blinded sessions (see methods)

² Geometric mean.

the allergens used for provocation. Criteria for inclusion in the study included forced expiratory volume in one second (FEV₁) of >75% predicted and bronchial hyperresponsiveness to histamine, defined as a provocative dose causing a 20% fall in FEV₁ (PD₂₀) of <1210 µg measured as previously described.²⁷ The study was approved by the local ethics committee and the Swedish Medical Products Agency (Läkemedelsverket). The patients gave informed consent.

STUDY DESIGN

All patients first underwent a screening allergen test to establish their current sensitivity to allergen. The PD₂₀ was determined by linear interpolation from the relation between cumulated dose of inhaled allergen and percentage change in FEV₁. Within six months of the screening challenge (mean interval 120 days, range 32–180) the double blind, placebo controlled, crossover drug trial was started. On two separate days the patients received either 750 mg BAYx 1005 or placebo four hours before allergen challenge. Patients reported fasting to the clinic at the same time of the day (07.30 hours) and ingested the tablets under supervision at about 08.00 hours.

Breakfast was given two hours later. The dose of BAYx 1005 used was the highest that had been cleared for human studies at the time the study was performed. The time for pre-administration was selected from data on peak plasma concentration (3–5 hours) and plasma half life (3–5 hours) previously determined in pharmacokinetic studies of the compound in man (data on file, Bayer AG, Germany). The compound BAYx 1005¹² was supplied by Bayer AG, Leverkusen, Germany, as 250 mg of a crystalline powder in coated tablets. The two blinded bronchoprovocations were separated by an interval of about four weeks (mean 27 days; range 20–56) and performed by inhalation of the same single dose of allergen on both occasions. The dose used in the blinded provocations (table 1) corresponded roughly to the PD₂₀ value obtained in the screening session.

ALLERGEN PROVOCATION AND STUDY DAY PROCEDURES

Bronchodilators were withheld for eight hours before the allergen challenges and were not

used during the study days. Short acting histamine H₁ receptor antagonists were not allowed for 48 hours before a study day. Long acting antihistamines, disodium cromoglycate, and non-steroidal anti-inflammatory drugs (NSAIDs) were not used for 10 days before a challenge session. Bronchoprovocation was performed by inhalation of allergen using a dosimeter controlled jet nebuliser (Spira Elektro 2, Respiratory Care Centre, Finland).⁶ Driven by compressed air at 7.5 l/min, the nebuliser generated an aerosol with a mass median particle aerodynamic diameter of 4.1 µm and the output was set to 7.1 µl per breath. Pulmonary function was measured as FEV₁ on a spirometer (Vitalograph MDI Compact, Förbandsmaterial, Sweden). Three concentrations of allergen extract (1000, 10 000, and 100 000 SQ/ml; SQ = standardised quality, the manufacturer's unit for allergen strength) were prepared by dissolving lyophilised powder in diluent. The extracts (Aquagen) and the diluent were from ALK Laboratories, Copenhagen, Denmark. By using the three concentrations and by varying the number of breaths from the nebuliser, the protocol used during the screening provocation resulted in approximately half log increments in the cumulated dose of allergen.

Baseline FEV₁ was defined as the best of three recordings made five minutes apart. Spirometric values were obtained at hourly intervals after drug administration and the FEV₁ value four hours after the drug was used as the baseline value for the ensuing allergen challenge. All challenges were preceded by inhalation of the diluent and, provided FEV₁ did not change by more than 10%, bronchoprovocation with allergen was started. Pulmonary function was measured every 15 minutes after inhalation of allergen until the FEV₁ had returned to within 10% of baseline, and thereafter hourly for up to six hours. For monitoring of late asthmatic reactions the patients measured their peak expiratory flow rate (PEFR) with a mini-Wright flow meter hourly after discharge from the hospital and until retiring to bed. The occurrence of a late airway reaction (LAR) was defined as a 20% drop in PEFR 3–12 hours after the early asthmatic reaction (EAR). The patients returned to the clinic the morning after each treatment day for physical examination, spirometric tests, and sampling of blood.

MEASUREMENTS OF URINARY LTE₄

Urine was collected at one hour intervals before drug or placebo, four hours after drug administration, and for up to six hours after the challenge. The samples were divided into different aliquots and stored separately at –20°C and –70°C. The concentration of LTE₄ was determined with a validated and semi-automated enzyme immunoassay method²⁸ and expressed as ng LTE₄/mmol creatinine.

MEASUREMENTS OF DRUG PLASMA LEVELS

Plasma samples were collected hourly before and after the challenge until the subjects left

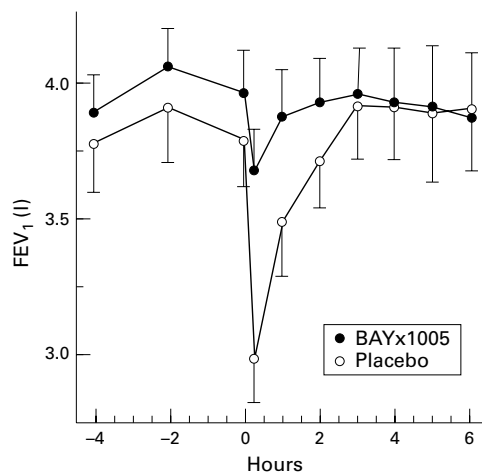


Figure 1 Group mean (SE) values for FEV_1 on the two study days. Placebo or 750 mg of the leukotriene biosynthesis inhibitor BAYx 1005 were ingested immediately after obtaining the first morning baseline value (-4 hours). The same single dose of allergen, corresponding to the PD_{20} value determined prior to the study, was inhaled at $t=0$ on both occasions. The peak fall in FEV_1 always occurred at the recording taken 15 minutes after the challenge.

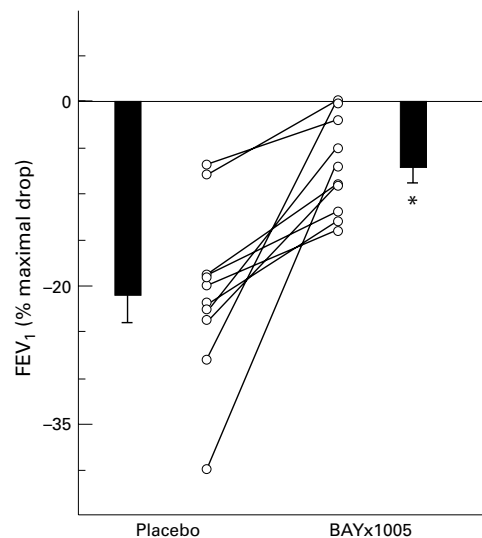


Figure 2 Peak fall in FEV_1 produced by inhalation of allergen following ingestion of placebo or 750 mg of the leukotriene biosynthesis inhibitor BAYx 1005 four hours before challenge. Individual results (○) and mean (SE) results for all 10 subjects on each occasion (■) are given. * $p<0.001$, Student's paired t test.

the clinic, transported on dry ice, and stored at below -15°C until analysis was performed at the human pharmacokinetic department of Bayer AG in Leverkusen, Germany. The HPLC method had a detection limit of $8\ \mu\text{g/l}$, a precision which varied from 7.6% to 12.7%, and an accuracy which deviated by a maximum of 3.8%.

ANALYSIS OF DATA

Geometric mean PD_{20} values were calculated on log transformed raw data. The area under the curves (AUC) for FEV_1 versus time and urinary LTE_4 versus time were calculated using the trapezoidal rule for integration. Results are presented as means (SE) and mean differences between treatments with 95% confidence intervals (CI). The data for pulmonary function and urinary levels of LTE_4 were found to be normally distributed (SigmaStat software for IBM PC, Jandel Scientific, USA). The Student's paired t test was used to compare group means.

Differences were considered to be significant when the p value was <0.05 . Period and carry-over effects of the drug treatments were analysed by the method of Hills and Armitage.²⁹

Results

Baseline pulmonary function was not significantly different on the two study days (fig 1), nor was there any significant change in FEV_1 during the four hours following drug administration (fig 1). There were no subjective or objective signs of drug related side effects after intake of 750 mg BAYx 1005.

There was good agreement between the fall in FEV_1 at screening and placebo for the eight subjects who inhaled the same dose of allergen on both occasions with a mean maximal fall of

26.5 (2.0)% and 23.5 (3.3)% for screening and placebo, respectively. For two subjects (4 and 10), the allergen dose selected for use in the double blind sessions (see methods) differed from the dose of allergen inhaled at screening. Subject 1 had late asthmatic reactions on screening and following placebo, but not after BAYx 1005.

The airway response to allergen was substantially inhibited when BAYx 1005 was given prior to challenge, both with respect to the amplitude of the fall in FEV_1 and the duration of the reaction (fig 1). Figure 2 shows the peak fall for each individual and the group means during the two sessions. The fall in FEV_1 was smaller after BAYx 1005 for all subjects. The mean peak fall after placebo was 21.0 (3.0)% compared with 7.1 (1.7)% after BAYx 1005. The mean difference between treatments was 13.9 (95% CI 7.0 to 20.8; $p<0.001$). Thus, the mean inhibition of the fall in FEV_1 by BAYx 1005 was 70.0 (7.0)%. The results were unrelated to the order in which treatments were given.

The mean area under the FEV_1 -time curve (AUC FEV_1) during the first hour after challenge was 5.3 (0.7) and 1.5 (0.4) for placebo and BAYx 1005, respectively. The mean difference between treatments was 3.8 (95% CI 2.2 to 5.4), $p<0.001$, which corresponds to a mean inhibition of the immediate airway response of 74 (10)%. The inhibition of the response during the first two hours after challenge was similarly calculated to be 63 (10.9)%.

Group mean baseline values for the urinary excretion of LTE_4 did not differ on the two study days (19.5 (6.7) and 23.5 (5.6) ng/mmol creatinine for placebo and drug, respectively), neither were there significant changes in the levels of urinary LTE_4 in the time between drug intake and allergen challenge. Thus, at the time of challenge urinary LTE_4 levels were 21.5 (4.7)

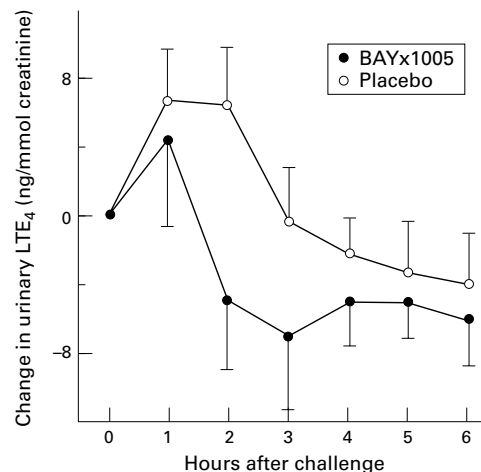


Figure 3 Group mean (SE) changes in post challenge urinary excretion of LTE_4 following placebo and the leukotriene biosynthesis inhibitor BAYx 1005. The concentration of LTE_4 in the sample of urine collected immediately prior to challenge (four hours after intake of placebo or drug) was selected as the reference on each occasion. For each individual this prechallenge concentration was subtracted from the value in the samples collected every hour after the provocation test. This produced a measure of net excretion after the challenge that was unrelated to each individual's baseline excretion of LTE_4 .

and 27.6 (4.7) ng/mmol creatinine following placebo and BAYx 1005, respectively.

The net urinary excretion of LTE_4 (value after challenge – prechallenge value) was increased after placebo (fig 3). This increase was significantly inhibited during the session when BAYx 1005 had been given. Thus, the mean AUC 0–2 hours after challenge for the net increase in urinary LTE_4 was 1.7 (0.9) for the placebo session and 0.4 (0.6) for the drug treatment session ($p < 0.05$), corresponding to an inhibition of 76%. The mean difference between treatments was 1.3 (95% CI –0.1 to 2.7). There were no differences in AUC for urinary LTE_4 between drug and placebo at time points later than three hours after challenge. Despite the similar degree of inhibition of urinary LTE_4 excretion and bronchoconstriction in the group, there was no correlation between the two responses in individuals ($r = 0.20$ for AUC LTE_4 at 0–2 hours versus AUC FEV_1 at 0–1 hours; $p > 0.58$).

Measurements of drug plasma concentrations confirmed the coding, with no detectable BAYx 1005 ($< 8 \mu\text{g/l}$) after placebo. On drug treatment days the peak concentration of BAYx 1005 was 10.5 mg/l (about $30 \mu\text{M}$) (range 5.3–19.8 mg/l; SE 2.3) and occurred at 3.5 (0.4) hours. The mean plasma half life of the drug was found to be 5.2 (1.5) hours. There was no correlation between the drug levels in the subjects and the degree of inhibition of either the airway response or the urinary excretion of LTE_4 ($r < 0.5$ for all tested hypotheses).

Discussion

The leukotriene biosynthesis inhibitor BAYx 1005 significantly inhibited allergen-induced

bronchoconstriction. Our findings with BAYx 1005 thus confirm previous indications^{25,26} that inhibitors of leukotriene biosynthesis attenuate allergen-induced bronchoconstriction. The inhibition of the early response in this study with BAYx 1005 was superior to that reported for the more short lived leukotriene biosynthesis inhibitor MK-886,²⁵ and was identical to that observed with another potent and long lived FLAP antagonist, MK-591.²⁶ The leukotriene biosynthesis inhibitors so far reported to protect against allergen-induced bronchoconstriction (MK-886,²⁵ MK-591,²⁶ and BAYx 1005) are all FLAP antagonists, whereas the drugs that have failed in allergen challenges of asthmatic subjects^{23,24} are directly acting 5-lipoxygenase inhibitors. Studies are required to establish if there is a real difference between the effects of FLAP antagonists and directly acting 5-lipoxygenase inhibitors on allergen-induced airway obstruction.

Furthermore, the extent of inhibition (about 70%) of the early asthmatic reaction with BAYx 1005 in this study and with MK-591 in the study by Diamant *et al*²⁶ is similar to the degree of protection which has been observed when allergen challenge has been performed in comparable study protocols after treatment with potent receptor antagonists of cysteinyl leukotrienes.^{3,4} Likewise, in a parallel study of the effect of BAYx 1005 on allergen-induced bronchoconstriction³⁰ a lower dose of BAYx 1005 (500 mg) was given for 3.5 days and caused about 60% inhibition of the maximal fall in FEV_1 during the early response. The similar effects of several structurally unrelated drugs that inhibit the action or formation of leukotrienes by different mechanisms therefore reinforces the suggestion that leukotrienes are major mediators of the early asthmatic response in humans. Secondly, the similar effects of leukotriene biosynthesis inhibitors and receptor antagonists of cysteinyl leukotrienes on the early response to allergen suggests that it is the bronchoconstrictive cysteinyl leukotrienes rather than the leucocyte attractant LTB_4 that mediate this particular response.

One of the main end points in this study was to measure LTE_4 in urine collected before, during, and after provocation in order to follow the degree of in vivo inhibition produced by BAYx 1005. As expected,^{19–22} the levels of LTE_4 were increased in the samples of urine collected within two hours after the placebo treated challenge with allergen. This increase was substantially inhibited by BAYx 1005. The magnitude of inhibition of net release (level after challenge – level before challenge) of LTE_4 (76%) was similar to the inhibition of the bronchoconstrictor response by the drug (70–74%, depending upon whether the peak fall in FEV_1 or AUC at 0–1 hours was measured). There is therefore good reason to believe that the inhibition by BAYx 1005 of the increase in urinary levels of LTE_4 seen after challenge reflected its ability to inhibit pulmonary formation of leukotrienes and consequently the allergen-induced airway obstruction. There was, however, no relation between the drug plasma concentration and the degree of in-

hibition of bronchoconstriction, nor between drug levels and the extent of inhibition of the allergen-induced urinary excretion of LTE₄, supporting previous suggestions that drug plasma levels of leukotriene antagonists or biosynthesis inhibitors do not correlate directly with drug effects on responses caused by the local release of leukotrienes in the airways.^{5,6,25,26}

In contrast to the inhibition of the allergen-induced enhanced urinary excretion of LTE₄, the prechallenge levels of urinary LTE₄ were unaffected by BAYx 1005. The method of measurement for urinary LTE₄ has been thoroughly validated, and the material determined with the present method is highly unlikely to be any other known metabolite of cysteinyl leukotrienes than LTE₄.²⁸ The finding therefore indicates that the overflow of urinary LTE₄ after allergen challenge reflects pulmonary formation of leukotrienes better than the baseline urinary excretion of LTE₄. This is intriguing and cannot be explained, but it may be important to consider in future work on the pharmacology of inhibitors of leukotriene synthesis.

Previous studies of the influence of anti-leukotrienes on allergen-induced airway obstruction have generated the hypothesis that individuals differ with respect to the participation of leukotrienes, because a proportion of the subjects have been described as non-responders.^{3-6,23} In this study, however, all subjects had a smaller and more short lived airway response after BAYx 1005 than after placebo. One reason for this uniform effect could be that the drug was ingested after overnight fasting and was well absorbed, as indicated by homogeneous plasma concentrations around 10 µM at the time of the challenge in all subjects. The half life of the drug was found to be 5.2 hours. Together, this indicates effective inhibition of leukotriene synthesis¹⁴ during the course of the reaction investigated. The findings thus lend strong support to the presence of a leukotriene component in all the subjects studied. The contribution of leukotrienes was most often substantial (>50% inhibition for eight of the 10 subjects, >70% inhibition for six). Nevertheless, there was some variability in effect (range of inhibition for fall in FEV₁ 37–100%) which, together with the lack of correlation between individual responses and degree of inhibition of urinary LTE₄, may indicate that the relative contribution of leukotrienes and other mediators might vary between individuals.

In conclusion, this study has shown that a single oral dose of the leukotriene biosynthesis inhibitor BAYx 1005 is sufficient to inhibit the early airway reaction to bronchial challenge with allergen. The inhibition was profound (about 70% of the response) and associated with a similar degree (about 75%) of inhibition of post-challenge excretion of urinary LTE₄. These findings add to the growing body of evidence in support of leukotrienes as important mediators in asthma, and suggest that this particular leukotriene inhibitor should be studied in other challenge models as well as in the treatment of asthma.

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