The leukotriene-receptor antagonist MK-0679 blocks airway obstruction induced by inhaled lysine-aspirin in aspirin-sensitive asthmatics

B. Dahlén*, M. Kumlin**, D.J. Margolskee+, C. Larsson*, H. Blomqvist*, V.C. Williams+, O. Zetterström*, S.-E. Dahlén++.

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ABSTRACT: Drugs which block the action or formation of the cysteinyl-leukotrienes (LTC_4, LTD_4 and LTE_4) inhibit asthmatic responses evoked by allergen, exercise and cold dry air. The purpose of this study was to determine whether the specific leukotriene-receptor antagonist MK-0679 could block the airway obstruction induced by aspirin (acetylsalicylic acid (ASA)) in aspirin-intolerant asthmatics.

Eight asthmatics (mean age 45 yrs), with an average history of asthma and ASA-sensitivity of about 10 yrs duration, were subjected to bronchial provocation with lysine-ASA. Baseline ASA-sensitivity was first determined in an open prestudy session by inhalation of cumulative doses of lysine-ASA to establish the dose of ASA decreasing forced expiratory volume in one second (FEV_1) by 20% (PD_{20}). Rechallenge with lysine-ASA was performed on two different occasions, 1 h after oral administration of placebo, or 750 mg of MK-0679, under double-blind conditions, in a randomized, cross-over design. Leukotriene formation was estimated by the measurement of urinary LTE_4.

The lysine-ASA challenge was highly reproducible (geometric mean for group PD_{20} being identical for the open prestudy and the placebo session), and was associated with a post-challenge increase in urinary LTE_4. In contrast, after MK-0679, there was a rightward shift in the dose response relationship for all eight subjects (median shift being 4.4 fold), with three of the subjects failing to produce a 20% decrease in FEV_1 despite inhalation of the highest dose of lysine-ASA feasible to deliver.

In conclusion, the leukotriene-antagonist MK-0679 substantially inhibited the airway response to inhalation of lysine-ASA, providing direct evidence that leukotrienes are mediators of ASA-induced bronchoconstriction.
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*Asthma and Allergy Division, Dept of Thoracic Medicine, Karolinska Hospital, Stockholm, Sweden. **Dept of Physiological Chemistry, and ++Physiology and Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, +Merck Research Laboratories, Rahway, NJ, USA.

Correspondence: B. Dahlén
Dept of Thoracic Medicine
Karolinska Hospital
S-104 01 Stockholm
Sweden.

Between 5 and 20% of adult asthmatics are intolerant to aspirin (acetylsalicylic acid (ASA)) and other non-steroidal anti-inflammatory drugs (NSAIDs) [1–3]. These patients are intolerant to all drugs inhibiting the cyclo-oxygenase enzyme which catalyses the formation of prostaglandins and thromboxane from arachidonic acid [4], but the precise mechanism behind this non-immunological hypersensitivity remains unknown. Nevertheless, the response to ASA in sensitive individuals has many similarities with an immediate hypersensitivity reaction. There are, indeed, indications that ASA-provocation may cause release of histamine [5], and tryptase [3], suggesting that mast cell activation is involved in the reaction. However, pretreatment with antihistamines fails to block ASA-induced airway obstruction

[6], indicating that other substances mediate the bronchoconstriction.

The leukotrienes (LT) were discovered in 1979, as a group of arachidonic acid products formed in many inflammatory cells, including eosinophils and mast cells (reviews [7, 8]). In particular, the mediator implicated in asthma and inflammation, slow reacting substance of anaphylaxis (SRS-A), was characterized as being composed of the cysteinyl-containing leukotrienes, LTC_4, LTD_4 and LTE_4. These three closely related leukotrienes are potent constrictors of human airways [9, 10] and it has recently been documented that cysteinyl-leukotrienes mediate airway obstruction induced by several triggers of asthma, including allergen [11–13], exercise [14, 15], and cold, dry air [16].

There are several observations which support the hypothesis that leukotrienes also may mediate ASA-induced bronchoconstriction. Firstly, inhalation of leukotrienes causes airway obstruction in ASA-sensitive asthmatics, thus meeting the first criteria required of a potential mediator ("appropriate biological activity"). In fact, it has been reported that this group of asthmatics may be exceptionally hyperresponsive to cysteinyl-leukotrienes, and perhaps especially to LTE_4 [17] Since ASA-provocation is associated with release of leukotrienes into body fluids, the second criteria of a putative mediator ("endogenous formation by appropriate stimulus") is also fulfilled. For example, leukotrienes have been detected in nasal lavage after oral [5], and nasal [18, 19], challenge with ASA. Furthermore, bronchial [20], and oral [21, 22], provocation with ASA caused airway obstruction associated with release of LTE_4 into the urine. Leukotriene E_4 is the final metabolite of cysteinyl-leukotrienes in the human lung [23], and it is rapidly excreted into the urine [24, 25]. At present, measurement of urinary LTE_4 has been found to serve as an indicator of pulmonary production of bronchoconstrictive leukotrienes [26].

Despite meeting these first two criteria of a mediator candidate, it has remained to be established that drugs which inhibit the action or release of leukotrienes can block ASA-induced airway obstruction. In one study, inhalation of a specific receptor antagonist of the cysteinyl-leukotrienes, SKF 104,353, caused only partial inhibition of the bronchoconstriction induced by oral ASA, in five out of six subjects, [27]. For the present study, MK-0679, an equally specific but more potent cysteinyl-leukotriene receptor antagonist [28], was given as a single oral dose before ASA-challenge, with the dose and the timing of drug administration carefully selected so as to provide maximal plasma levels, and presumably optimal antagonism of endogenous cysteinyl-leukotrienes at the time of the provocation. Furthermore, since oral provocation with ASA causes both systemic and pulmonary reactions, bronchial provocation with inhaled lysine-ASA (a more soluble form of ASA) was selected to specifically address the role of leukotrienes in the airway response [29].

The hypothesis that leukotrienes are mediators of the response to ASA would, thus, be confirmed if the leukotriene-receptor antagonist MK-0679 could blunt the response to lysine-ASA. The protocol for bronchial provocation with ASA involved cumulative challenge with increasing doses of lysine-ASA until forced expiratory volume in one second (FEV_1) had dropped by 20% or more. Since blockade by a competitive antagonist can be surmounted by higher doses of the agonist, it follows that in the presence of the antagonist, higher doses of ASA should be required to produce the 20% drop in FEV_1. Using a similar cumulative challenge protocol, we have previously reported that compared with placebo, the leukotriene-antagonist ICI-204,219 produced a significant increase in the dose of allergen required to achieve bronchoconstriction [11]. We also observed that the allergen-induced urinary excretion of LTE_4 was higher in the presence of the leukotriene antagonist [20], which was interpreted as evidence of increased formation of

leukotrienes when the dose of the provocative agent was higher. Therefore, in order to determine whether similar dose-dependent release of leukotrienes could be demonstrated in ASA-induced airway reactions, measurements of urinary LTE_4 were made before and after the ASA bronchoprovocations.

Methods

Patients

Eight nonsmoking asthmatics (for characteristics, see table 1) were selected for the study on the basis of ASA-intolerance, documented by previous oral or bronchial challenge, or an unequivocal history. The study was approved by the local Ethics Committee, and the patients gave informed consent. For inclusion in the study, regular treatment with systemic corticosteroids, inhaled cromolyn, oral bronchodilators or long-acting H_1-antagonists was not allowed, whereas it was acceptable to use inhaled bronchodilators, oral theophylline, or inhaled corticosteroids (daily dose of budesonide not exceeding 1,200 µg).

Study design

All patients first underwent a prestudy challenge, involving inhalation of rising doses of lysine-ASA to determine the current provocative dose of ASA [µmol] producing a 20% fall in FEV_1 (PD_{20} FEV_1). After the prestudy challenge (average interval was 33 days, range 10–69 days), the double-blind, placebo-controlled, crossover drug trial started. On two separate days, the patients received either 750 mg of MK-0679 or placebo tablets one hour before ASA challenge was initiated. Patients always reported to the clinic at the same time of day (i.e. 7.30 am); MK-0679 was taken on an empty stomach, and blood for assay of MK-0679 was drawn 15 min before and at 60 and 120 min after drug intake. The two bronchoprovocations were separated by an interval of 7–14 days, and the same protocol for ASA dose increments was used at both occasions. For each challenge, the starting ASA dose was selected as 0.5 to 1 log below the PD_{20} FEV_1 determined with the same protocol at prestudy. Pulmonary function was followed for 7 h after challenge. In addition, urine samples were obtained at hourly intervals throughout each study day, divided into aliquots which were kept in sealed plastic containers and stored in separate -20 and -70°C freezers, until LTE_4 and creatinine were assayed as described below. If considered necessary, in the case of pronounced and persistent airway obstruction, rescue treatment with inhaled β-agonist and/or ipratropium bromide was given, and inhaled budesonide administered as anti-inflammatory supplementation. Patients were sent home for the night, but returned to the clinic the next morning for general follow-up, including physical examination, spirometry, and blood sampling for routine haematology and blood chemistry.

Table 1. – Patient characteristics

Pt. no.	Sex	Age yrs	ASA-sensitivity	History of yrs Asthma	Nasal symptoms	Prestudy FEV₁ % pred	Prestudy ASA PD₂₀ μmol	Medication during study
61	M	60	14	16	12	65	50	IB, IS, OT
62	M	29	4	4	5	84	3	IB, IS, OT
63	M	49	4	5	5	60	11	IB, IS
64	F	43	9	9	20	98	126	IB
65	M	53	9	9	9	99	92	IB,IS
66	M	57	1	1	11	98	8	IB
67	F	31	4	4	5	93	307	IB, IS
68	F	35	26	26	11	73	70	IB, IS
Mean:		45	9	9	10	84	83	IB: n=8
Range:		29–60	1–26	1–26	5–20	60–99	3–307	IS: n=6
								OT: n=2

IB: inhaled beta-stimulant; IS: inhaled steroid; OT: oral theophylline; ASA: acetylsalic acid (aspirin); FEV₁ forced expiratory volume in one second; PD₂₀: provocative dose producing a 20% fall in FEV₁.

Throughout the study, patients continued their regular asthma therapy (table 1), except for withholding oral theophylline for 24 h, inhaled steroids for 12 h and inhaled β-agonists for 8 h before a visit to the clinic. One patient (subject no. 68) was allowed to inhale β-agonist (terbutaline 0.5 mg) early in the morning on the separate treatment days, but this occurred at the same time (3 h before the bronchoprovocation) on each study day.

Protocol for bronchial challenge with ASA

Bronchoprovocation was performed by inhalation of lysine-ASA (Aspira Medical, Borlänge, Sweden) as described previously [29], except that a dosimeter-controlled jet nebulizer (Spira Elektro 2, Respiratory Care Center, Hameenlinna, Finland [30]), was used to permit determination of PD_{20}. Driven by compressed air at 7.5 $l \cdot min^{-1}$ the nebulizer generated an aerosol with a mass-median particle aerodynamic diameter of 4.1 μm; with a 0.8 s nebulization period the output was 10.3 $\mu l \cdot breath^{-1}$. Pulmonary function was measured as FEV_1, on a spirometer (Vitalograph MDI Compact, Förbandsmaterial, Sweden), using the best of three efforts. Baseline FEV_1 was defined as the mean of two recordings, 15 min apart. Bronchoprovocation was performed starting with inhalation of diluent and, providing FEV_1 did not change by more than 10%, followed by inhalation of increasing doses of ASA every 30 minutes. Spirometry was obtained at 10, 20 and 30 min after each dose of ASA, and the challenge was stopped when FEV_1 had fallen ≥20% from the post-diluent baseline, or the maximum dose of ASA had been reached. For safety reasons, in the case of a decrease in FEV_1 between 15–20%, indicating the development of a positive reaction, another 10 min interval followed and then, provided that FEV_1 was still between 15–20% below baseline, a decision was made whether to give the next dose or to repeat the previous one. After a positive reaction, spirometry was followed every 15 min until FEV_1 had returned to within 10% of the post-diluent baseline and thereafter hourly. Dose-response relations for

the cumulated dose of ASA were constructed, and used for calculation of the PD_{20}.

The solutions for bronchoprovocation were freshly prepared on each day by dissolving crystalline lysine-ASA (Aspisol®, Horby Bayer AG, Germany) in 0.9 sodium chloride, to produce a stock solution (1 M= 180 $mg \cdot ml^{-1}$ of ASA) and a tenfold dilution. By using these two solutions, and by varying the number of breaths (1, 2 and 7) from the nebulizer, a protocol was developed creating approximately half-log increments in the cumulated dose of ASA (1, 3, 10, 30, 100, 300 and 600 μmol).

Analytical procedures

Urinary LTE_4 was determined in the hourly samples by radio-immunoassay (RIA) essentially as described previously [20], except that ^3H-LTE_4 was used as tracer, and LTE_4 as standard. The concentration of immunoreactive LTE_4 was expressed as $ng \cdot mmol^{-1}$ of creatinine (measured colorometrically). The detection limit for LTE_4 was 7.5 $pg \cdot ml^{-1}$ urine, which during normal diuresis gives rise to a threshold for detection of 1–5 $ng \cdot mmol^{-1}$ creatinine (= 10–45 $pg \cdot mg^{-1}$ creatinine). The amount of LTE_4 in the sample collected during the first hour after entering the clinic (before intake of placebo or drug), was used as prechallenge value. The sample with highest LTE_4 content of those collected after the airway response to ASA had appeared, was used as postchallenge value. For calculation of net release during the provocation, the basal prechallenge values were subtracted from the postchallenge concentrations. All samples were analysed within three months after the last provocation. We have previously reported that samples stored at -20°C produced consistent results for at least 10 months [20].

For comparison of RIA determinations before and after high performance liquid chromatography (HPLC) separation, urinary samples from four of the provocation sessions (10 samples from each session) were randomly selected for further analysis. Briefly, 4 ml of each sample was spiked with ^3H-LTC_4 (New England Nuclear, Boston, MA, USA, $Ci \cdot mmol^{-1}$; 10,000 dpm). The pH was

adjusted to around 4 with formic acid and 1 vol. of methanol was added. The samples were left at -20°C for 1 h prior to removal of precipitated material by centrifugation. The methanolic supernatants were extracted on Chromabond® C_{18} columns (0.5 g, Macherey-Nagel, Düren, Germany), which were washed with water, methanol/water (1:1), and finally eluted with pure methanol. The eluates were taken to dryness under a stream of N_2 and the residues were redissolved in HPLC mobile phase. The samples were further subjected to reverse phase-HPLC (RP-HPLC) as described previously [23]. Fractions corresponding to the retention volume of LTE_4 were analysed with RIA. Losses during purification were corrected for by the internal standard ^3H-LTC_4, which was measured by liquid scintillation counting of appropriate HPLC fractions. The RIA of unpurified urine and samples subjected to HPLC were always performed on the same occasion for each subject.

The plasma concentration of MK-0679 was measured by HPLC as described previously [31].

Statistics

The analysis of variance model of Grizzle was used to compare means between treatment groups. The ANOVA model contained terms for sequence, patient within sequence, period and treatment. The normality assumption of the ANOVA was tested and verified with the Shapiro-Wilk statistic. Hartley's maximum F-test on the variance of the residuals was used to verify the homogeneity of variance assumption. All tests were two-tailed at $\alpha=0.05$. Results are generally reported as mean±standard error (SEM), and considered to be significantly different if $p<0.05$.

Calculations of group mean or median PD_{20} values were performed on log transformed raw data. The repeatability of the challenge procedure was evaluated by comparing logarithmically transformed PD_{20} values at prestudy and placebo sessions, using the method described by BLAND and ALTMAN [32]. Briefly, the difference in PD_{20} values at the two occasions was plotted against their mean.

Results

Drug tolerability

All patients completed the study without subjective or objective signs of side-effects related to MK-0679.

Effect of MK-0679 on bronchoconstriction induced by ASA

Figure 1 shows an example of the time course of the airway response to inhalation of progressively increasing doses of ASA and the levels of urinary LTE_4, in one of the subjects. The response to ASA was highly reproducible in this group of patients, with the geometric means of PD_{20} values for ASA being 37±2 and 34±2 µmol on prestudy and placebo days, respectively. The 95% confidence interval for the difference in results between the two challenges was 0.6–1.8 fold. In line with previous reports [6, 29], the immediate bronchoconstriction to ASA was not followed by a late phase reaction, or by the appearance of extra-pulmonary symptoms.

Pretreatment with MK-0679, 750 mg, caused a distinct rightward shift of the dose-response relation for ASA in all eight individuals, when compared to placebo (fig. 2). In fact, three of the subjects did not respond with the stipulated 20% decrease in FEV_1 to the highest dose of ASA that was possible to administer. In order to include these three individuals in the estimation of the median shift of PD_{20} in the group, their PD_{20} values were set as equal to the highest cumulated dose of ASA given in the presence of the antagonist. Despite this underestimate of the influence of the drug, MK-0679 caused a highly significant ($p<0.001$) increase of the PD_{20} for ASA in the group (fig. 3), with the median shift being 4.4 fold (after log transformation). There was no difference in baseline prechallenge FEV_1 between the placebo and MK-0679 treated day (means±SEM: 2.8±0.3 and 2.9±0.2 l for placebo and MK-0679, respectively), and there was no drug-related change in the baseline FEV_1 during the hour which passed between drug intake and the start of the provocation (means±SEM % change in FEV_1 from predrug being 2±4 for placebo and 4±8% for MK-0679, NS).
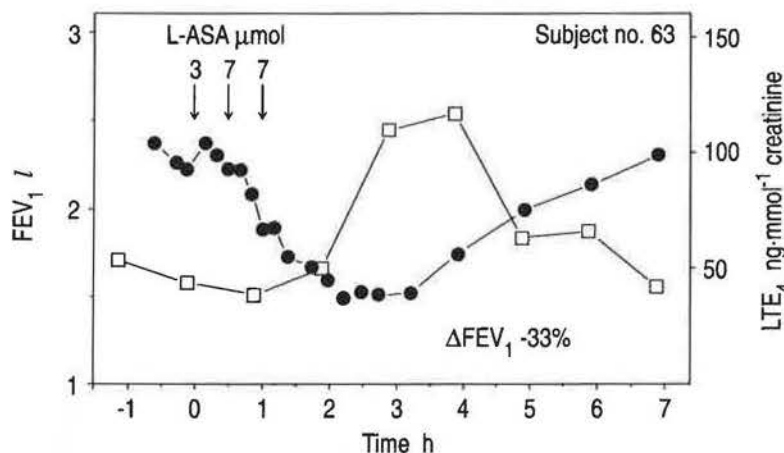

Fig. 1. – Time-course of ASA bronchoprovocation in one of the subjects (no. 63) on the placebo study day. Pulmonary function was measured as FEV_1 (●——●). After inhalation of diluent, cumulative doses of lysine-ASA (L-ASA) were inhaled every 30 min until FEV_1 had fallen by 20% from post-diluent baseline. Urinary exretion of LTE_4 (□——□) was determined by RIA in samples collected hourly during the observation period. ASA: acetylsalicylic acid (aspirin); FEV_1: forced expiratory volume in one second; LTE_4: leukotriene E_4; RIA: radio-immunoassay.
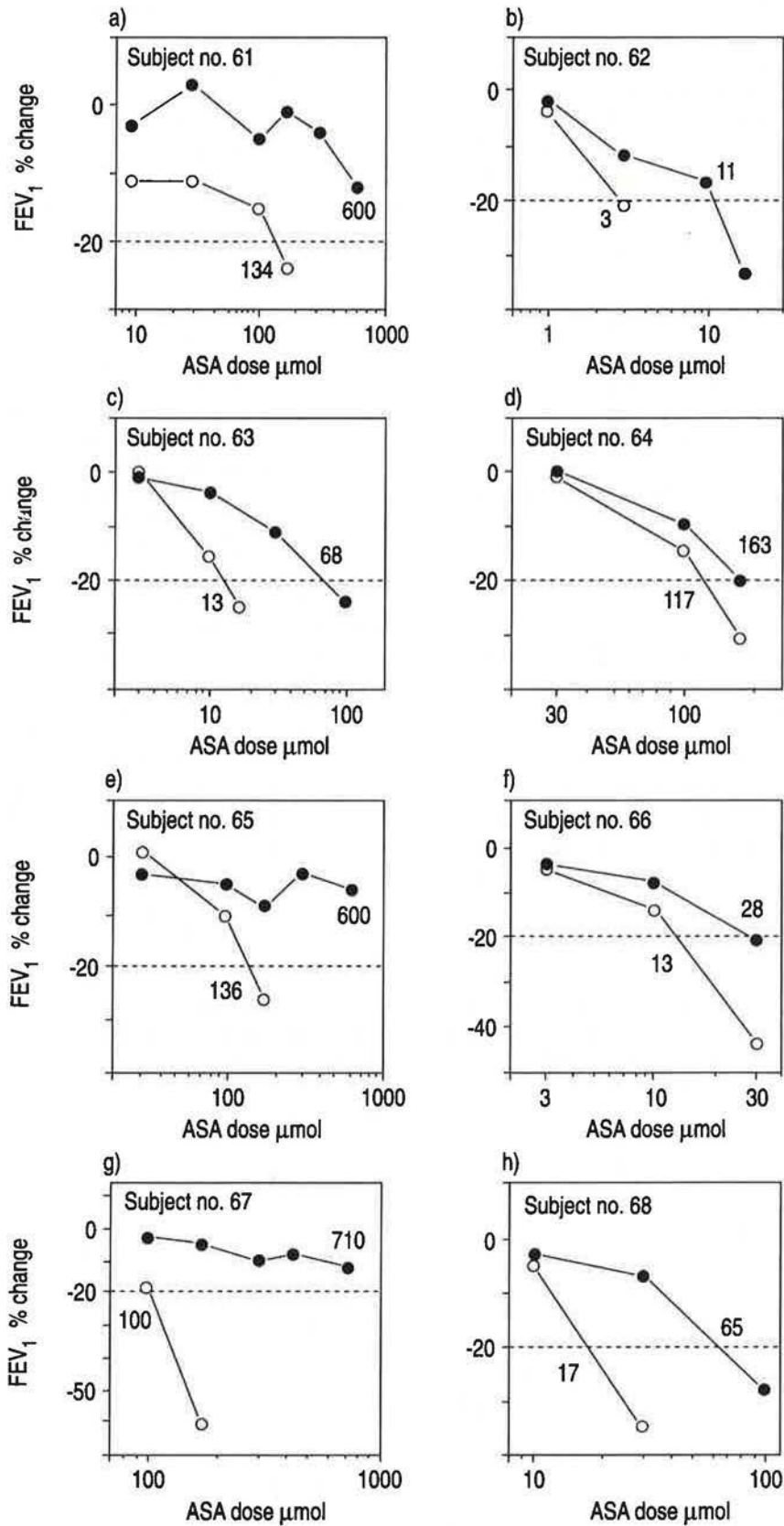
Fig. 2. – Dose-response relations for cumulative doses of inhaled ASA on placebo (O——O), and MK-0679 (●——●) treated days in the 8 subjects (a–h). Each point represents the percentage change in the FEV₁ from zero (baseline) for every dose of inhaled lysine-ASA. The PD₂₀ values calculated from each curve are shown on the graphs. Note that patients no. 61, 65 and 67 did not reach a 20% fall in FEV₁ after MK-0679 treatment, and the value in these cases represent the total dose of ASA inhaled. PD₂₀: provocative dose producing a 20% fall in FEV₁. For further abbreviations see legend to figure 1.

Upon dismissal from the clinic (6–7 h after the start of the provocation), the FEV_1 had returned to baseline values, and there were no differences in this respect between the placebo and drug-treated groups.

In addition, after treatment with MK-0679, the maximal fall in FEV_1 (within 90 min after the last dose of ASA) was significantly less than after placebo (29±6% *versus* 42±5%).
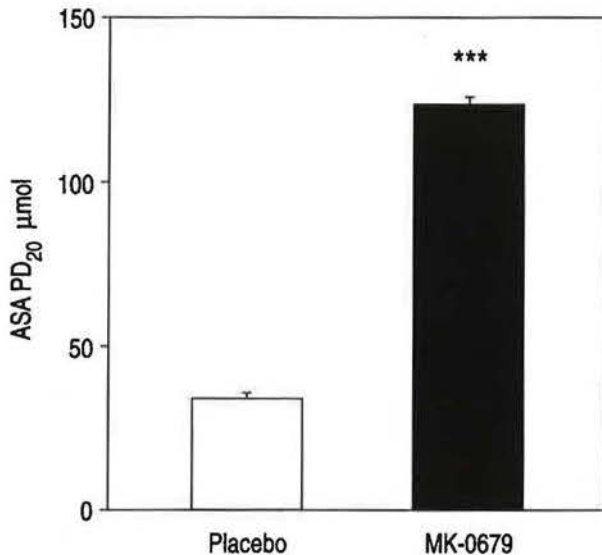

Fig. 3. – A conservative estimate of effect of MK-0679 on PD_{20} for ASA in the group as a whole. Geometric mean±SEM for ASA PD_{20} after placebo (open bar), and MK-0679 (solid bar), with the PD_{20} for patients no. 61, 65 and 67 after drug being set equal to the highest inhaled dose. ***: p≤0.001. For abbreviations see legend to figure 1 and 2.

As a corollary, there was a tendency for more rescue treatments during the placebo sessions, when three subjects (nos 62, 66 and 67) were given inhaled β-agonist and ipratropium bromide, and two of them, on clinical grounds, also received budesonide after the termination of the provocation. During the MK-0679 study days, two of the same subjects (nos 62 and 66) were again rescued with bronchodilators, but inhaled steroids were not administered.

Measurement of MK-0679 plasma levels confirmed the correctness of the coding. The levels at 1 and 2 h post-drug were equal (39±9 and 42±8 $\mu g \cdot ml^{-1}$), indicating that peak levels were reached by the time the provocations were started. These findings are also in accordance with available pharmacokinetic data [31]. When the results were analysed on an individual basis, there was, however, no clear-cut correlation between drug concentration and the actual shift in PD_{20} or the maximal drop in FEV_1.

Urinary excretion of LTE_4

The basal excretion of LTE_4 was not changed by MK-0679; one hour post drug intake (but before the start of the challenge), the urinary concentrations of LTE_4 were 106±9 and 95±9 $ng \cdot mmol^{-1}$ creatinine after placebo and MK-0679, respectively. The bronchoconstrictor response to ASA was associated with increased concentrations of immunoreactive LTE_4 in the urine (figs 1 and 4a), with the peak concentration found in samples collected 1–4 h after administration of the last dose of ASA. The ASA-induced release of LTE_4 into the urine was found to be higher after MK-0679 than after placebo (fig. 4a).
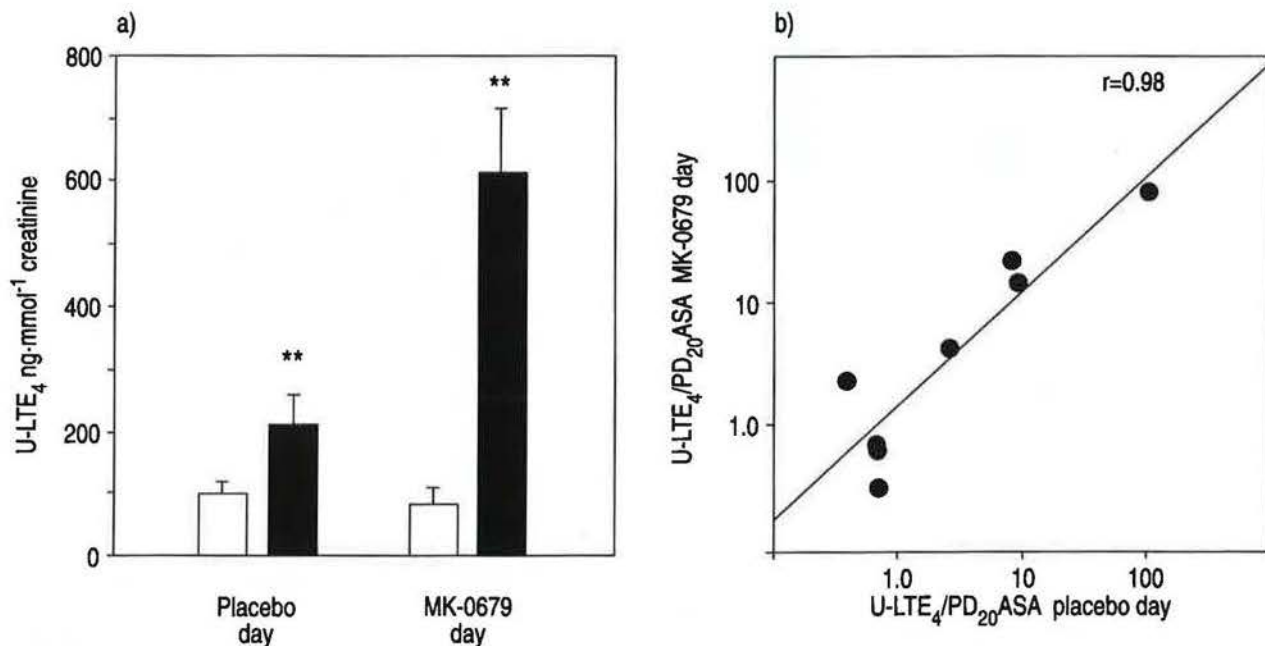

Fig. 4. – a) Urinary levels of immunoreactive LTE_4 (U-LTE_4) before (open bar) and after (solid bar) challenge with ASA for placebo and MK-0679 treated days. ASA induced a significant release of U-LTE_4 which was even more pronounced on the MK-0679 study day. **: p≤0.01. b) Comparison of individual ratios between U-LTE_4 release (postchallenge minus prechallenge) and PD_{20} ASA on the placebo and MK-0679 study days. On the two days, all subjects expressed a very similar ratio between the extent of LTE_4 excretion and the degree of stimulation (i.e. the dose of ASA), thus supporting a dose-response relationship for LTE_4-release. For abbreviations see legend to figures 1 and 2.

However, the ratio between urinary levels of immunoreactive LTE_4 (U-LTE_4) and PD_{20} for ASA was found to be the same for each subject at the two challenge sessions (fig. 4b). A similar high correlation was also found if the ratio between U-LTE_4 and total cumulated dose of ASA was calculated for the two sessions (r=0.93).

As a technical note, LTE_4 was regularly assayed directly by RIA of unpurified urine (see methods section). The validity of this approach, omitting the HPLC separation, was verified by performing RIA measurements on samples (more than 40) from four of the provocation sessions both before and after purification by HPLC. After correction for losses during purification and separation, as estimated by recovery of the internal standard (^{3}H-LTC_4), the concentrations of immunoreactive LTE_4 determined before and after HPLC correlated very well (r=0.88). Consequently, as shown in figure 5, the same information was obtained by using the two approaches. By performing the alternative statistical analysis of method reliability by BLAND and ALTMAN [32], additional evidence was provided (not shown) to support the agreement between measurements of LTE_4 in the unpurified and purified urine.
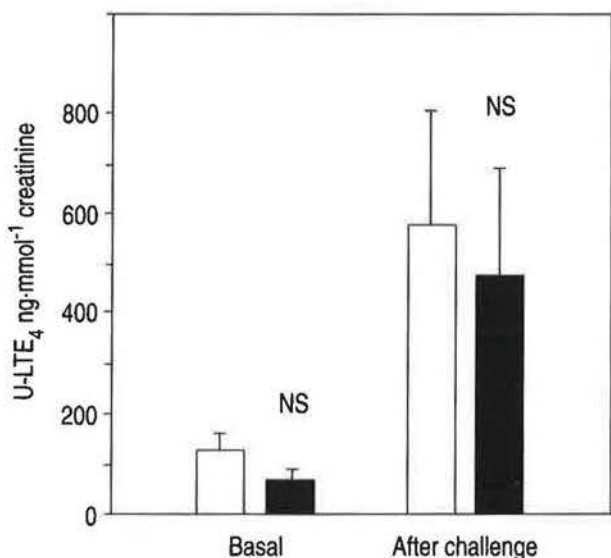

Fig. 5. – Urinary samples from four provocation sessions were analysed for LTE_4 by RIA, both in unpurified urine (open bar) and after purification by HPLC (solid bar). The amount of LTE_4 measured with the alternate approaches did not differ significantly. HPLC: high performance liquid chromatography. For further abbreviations see legend to figure 1.

Discussion

Oral pretreatment with the specific and potent receptor antagonist of the cysteinyl-leukotrienes, MK-0679 [28], caused a highly significant inhibition of the airway response to inhalation of cumulative doses of ASA. In comparison with placebo, all eight subjects required higher doses of ASA to reach the stipulated degree of bronchoconstriction in the presence of the competitive receptor antagonist. Together with previous demonstrations that inhalation of leukotrienes induces airway obstruction in ASA asthmatics [17], and that leukotrienes

are released in response to ASA provocation [5, 18–22], as also documented in this study, it is therefore possible to conclude that the cysteinyl-leukotrienes indeed fulfil the criteria of being true mediators of ASA-induced airway obstruction in ASA sensitive asthmatics. As mentioned in the introduction, treatment with antihistamines has no significant effect on the airway response to ASA [6]. Therefore, the cysteinyl-leukotrienes appear to be the most important mediators so far identified as being involved in ASA-induced bronchoconstriction.

A previous challenge study in ASA-sensitive asthmatics reported a relatively small and variable inhibition of the airway response to ASA by the leukotriene antagonist SKF 104,353 [27]. Probably, this may be related to a less effective degree of leukotriene-antagonism (tenfold displacement of LTD_4 dose-response curve) with that particular dose of the inhaled antagonist SKF 104,353 [33]. In the present study, the antagonist MK-0679 was administered orally in a dose which produced plasma levels around 40 µg·ml⁻¹; this plasma concentration is approximately tenfold higher than that expected to cause a 25 fold displacement of the dose-response relation for inhaled LTD_4 [34]. Alternatively, since the study with SKF 104,353 involved oral provocations with ASA, it is possible that this systemic challenge causes bronchoconstriction related to mechanisms other than those due to the direct effect of mediators released locally in the airways.

It has recently been reported that leukotriene antagonists or lipoxygenase inhibitors [35–37] may cause bronchodilation in asthmatics, an effect that is not seen in normal volunteers [38]. Since the leukotriene antagonists lack nonspecific bronchodilator action [28, 39], these clinical observations in fact provide circumstantial evidence of endogenous leukotriene production in inflamed asthmatic airways. Bronchodilation in response to MK-0679 would of course be a possible confounding factor in the interpretation of the results of the present provocation study. However, as reported above, bronchodilation did not occur with MK-0679 in the hour that passed between capsule intake and the start of the provocation. In another group of ASA-sensitive asthmatics, we have recently characterized the bronchodilation response to a higher dose of MK-0679 (manuscript under review). When such a response occurred, it was in the patients with the most severe airway disease. The response always appeared within 30 min after drug administration and was close to maximal after 60 min. Therefore, there is no indication that bronchodilation contributed to the inhibition of provocation response reported in this study.

The present study also supports and extends previous indications [20–22] that the urinary excretion of LTE_4 is increased in response to challenge with ASA in ASA-sensitive asthmatics. Furthermore, pretreatment with MK-0679 allowed an increase in the dose of ASA (stimulus) and the excretion of LTE_4 (response), supporting a dose-dependent release of LTE_4 by ASA in ASA-sensitive asthmatics. The basal urinary excretion of LTE_4 was, however, not changed by the antagonist. In a placebo-controlled trial in allergic asthmatics, we have documented that the leukotriene antagonist ICI-204,219 likewise

increased the PD_{20} for allergen and the post-allergen excretion of LTE_4 into the urine [20]. Considered together, these data support the likely hypothesis of dose-dependent excretion of LTE_4 in response to different provocative stimuli. The present observation that the ratios between the dose of ASA (expressed as the PD_{20} value, or the total dose of ASA inhaled) and the response (*i.e.* urinary excretion of LTE_4), were almost identical for each individual at both sessions (fig. 4b), would seem to further support the hypothesis that provocation-induced release of leukotrienes is dose-dependent.

The basal levels of urinary LTE_4 in this study (80–100 $ng \cdot mmol^{-1}$ creatinine) are in the same range as we have previously reported for aspirin-sensitive asthmatics [20]. Therefore, together with the studies directly comparing aspirin-sensitive and aspirin-tolerant asthmatics, [21, 20, 40], the data in this investigation lend indirect support to the concept that aspirin-sensitive asthmatics have higher basal levels of urinary LTE_4 than other asthmatics.

Concerning the technical aspects of the measurements of urinary LTE_4, RIA of unpurified urine gave results that correlated closely with those obtained by the more time-consuming approach of extraction, followed by HPLC separation [20, 41, 42]. We have previously documented that the presently used RIA specifically measures LTE_4 in the urine [20]. Although validation by HPLC must be carried out on selected samples, the strategy to directly measure LTE_4 in unpurified urine by RIA should be useful in clinical studies. In addition, when using an immunoassay (RIA or eletroimmunoassay (EIA)), it is always a possibility that running samples through several steps of purification will in fact introduce factors that may interfere with the assay [43].

As a further methodological point, it is well-known that sensitivity to ASA and other NSAIDs varies with time in ASA-sensitive asthmatics, and a state of desensitization may even be achieved by repeated dosing with ASA [2, 3]. Therefore, it was encouraging to find that the present protocol for repeated bronchial provocations with lysine-ASA proved to have the high reproducibility required for a double-blind, pharmacological trial. In fact, using the method of BLAND and ALTMAN [32] to evaluate the reproducibility, 95% of the differences between the repeated provocations (prestudy and placebo study day) were less than two standard deviations, which is the definition of repeatability adopted by the British Standards Institution. The reproducibility of provocations with lysine-ASA was also shown by PHILLIPS *et al.* [6]. If anything, our reproducibility was slightly higher, with the 95% confidence interval for variation always being less than a doubling dose of ASA (0.6–1.8 fold). By performing the challenges at an interval of 7–14 days, as was done both in this study and the study of PHILLIPS *et al.* [6], the possible effect of time-dependent variations in ASA-sensitivity may be minimized.

It is now established that cysteinyl-leukotrienes are significant mediators of allergen-induced bronchoconstriction in man [11–13]. Inhibition of leukotrienes will also protect against airway obstruction induced by exercise [14, 15], or inhalation of cold, dry air [16], and the present data document leukotriene involvement in ASA-induced

airway obstruction. On the basis of our present knowledge, it is reasonable to generalize and suggest that release of leukotrienes may be a final common path for many provocative stimuli that cause asthmatic reactions. It has recently been reported that ASA-sensitive asthmatics, as a group had higher baseline excretion of $U-LTE_4$ than other asthmatics [20, 21, 40], and therefore, together with the present findings, it is suggested that ASA-sensitive asthmatics may be one important group in which to test the therapeutic potential of drugs which inhibit leukotrienes.

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