Effect of the 5-lipoxygenase inhibitor ZD2138 on aspirin-induced asthma

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Abstract

Background – The cysteinyl leukotrienes may play a central part in the mechanisms of aspirin-sensitive asthma. Previous work has shown that individuals with aspirin-sensitive asthma have high basal urinary LTE_4 levels which increase further upon aspirin ingestion, and that sulphidopeptide leukotriene receptor antagonists attenuate aspirin-induced airflow obstruction. If the cysteinyl leukotrienes cause aspirin-induced asthmatic reactions, inhibition of the 5-lipoxygenase pathway should prevent aspirin-induced bronchospasm. This hypothesis has been tested with ZD2138, a specific non-redox 5-lipoxygenase inhibitor.

Methods – Seven subjects (four men) with aspirin-sensitive asthma with baseline FEV_1 values >67% were studied. ZD2138 (350 mg) or placebo was given on two separate occasions two weeks apart in a randomised double blind fashion. A single dose of aspirin was administered four hours after dosing and FEV_1 was measured for six hours. Inhibition of the 5-lipoxygenase pathway by ZD2138 was assessed by measurements of urinary LTE_4 levels and ex vivo calcium ionophore stimulated LTB_4 generation in whole blood, before administration of drug or placebo and at regular time intervals after dosing and aspirin administration.

Results – ZD2138 protected against the aspirin-induced reduction in FEV_1 with a 20·3 (4·9)% fall in FEV_1 following placebo compared with 4·9 (2·9)% following ZD2138. This was associated with 72% inhibition of ex vivo LTB_4 generation in whole blood at 12 hours and a 74% inhibition of the rise in urinary LTE_4 excretion at six hours after aspirin ingestion.

Conclusions – In aspirin-sensitive asthma the 5-lipoxygenase inhibitor ZD2138 inhibits the fall in FEV_1 induced by aspirin and this is associated with substantial inhibition of 5-lipoxygenase.

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Asthmatic patients with aspirin-sensitive asthma[1 2] are a readily identifiable group of subjects who develop bronchospasm on ingestion of aspirin or non-steroidal anti-inflammatory drugs. Other aspirin-induced symptoms in these subjects include rhinorrhoea, nasal and ocular congestion, generalised flushing, nausea, vomiting, headache, and abdominal cramps. Characteristically these subjects have persistent nasal polyposis, rhinorrhoea, nasal congestion, anosmia with loss of taste, and frequent exacerbations of asthma.[3–5]

Arachidonic acid released from cell membrane phospholipids by phospholipase A_2 may be further metabolised by the cyclooxygenase pathway to prostaglandins and thromboxane A_2 or by the 5-lipoxygenase pathway to leukotrienes.[6] The initial leukotriene formed by the action of 5-lipoxygenase is LTA_4, which is then further metabolised by an epoxide hydrolase to LTB_4 or by a glutathione S-transferase termed LTC_4 synthase[7] to LTC_4, which is further cleaved to LTD_4 by gamma-glutamyl transpeptidase and to LTE_4 by a dipeptidase. The cysteinyl leukotrienes LTC_4, LTD_4, and LTE_4 are potent spasmogens for airway smooth muscle[8] and comprise the activity previously recognised as slow reacting substance of anaphylaxis.[9]

The mechanism of aspirin-sensitive asthma remains unresolved, but it is known that the ability of drugs to cause symptoms in aspirin-sensitive asthma is related to their potency as cyclooxygenase inhibitors.[10] It has been proposed that blockade of cyclooxygenase activity by these agents leads to diversion of arachidonic acid metabolism from the cyclooxygenase to the 5-lipoxygenase pathway with consequent increased leukotriene production.[11] The evidence for the involvement of the cysteinyl leukotrienes in aspirin-sensitive asthma is compelling. Subjects with aspirin-sensitive asthma have six times higher basal levels of urinary LTE_4 than aspirin-tolerant asthmatic and non-asthmatic subjects.[12] Furthermore, aspirin challenge of subjects with aspirin-sensitive asthma, which leads to bronchoconstriction, is accompanied by a mean fourfold increase in urinary LTE_4 excretion.[12] No clinical symptoms or increase in urinary LTE_4 levels are detected in non-aspirin-sensitive asthmatic individuals following aspirin ingestion. Additionally, there is increased target organ sensitivity to the bronchospastic effects of inhaled LTE_4 in subjects with aspirin-sensitive asthma compared with aspirin-tolerant asthmatic subjects.[13] Previous studies have shown the efficacy of cysteinyl leukotriene receptor antagonists in the mechanisms of aspirin-sensitive asthma.[14 15] If leukotrienes are critical in the asthmatic response provoked by aspirin, one might expect an inhibitor of 5-lipoxygenase to block aspirin-sensitive asthma. We have tested this hypothesis with ZD2138, a novel non-redox lipoxygenase inhibitor[16] which is well tolerated and, at single oral doses of 350 mg or greater, inhibits ex vivo LTB_4 synthesis in whole blood for 24 hours.[17]

Methods

SUBJECTS

Seven non-smoking subjects (three women) of mean age 42 (range 35–52) years with aspirin-sensitive asthma were studied (table 1). Their resulting mean (SE) forced expiratory volume in one second (FEV_1) was 2·57 (0·17) l (mean predicted 86%; range 67–105%). All subjects also suffered with symptoms of rhinosinusitis comprising nasal congestion, rhinorrhoea, and varying degrees of dysosmia and loss of taste. The study was approved by the Guy's Hospital ethics committee and written informed consent was obtained from each subject.

STUDY PROTOCOL

Subjects were screened for aspirin sensitivity if they had a history of worsening asthma on aspirin ingestion. They were studied on three days, each separated by two weeks. On day 1 a medical history was taken and the subjects underwent general physical examination with an ECG. Blood was taken for a full blood count, clotting studies, estimation of electrolytes and urea, and liver function tests. Urine was examined by microscopy and tested for glucose, protein, and blood. Oral aspirin challenge was then performed with incremental doses of aspirin at two-hourly intervals, starting with 30 mg. The dose of aspirin resulting in a 20% or greater fall in FEV_1 within two hours of ingestion was considered the threshold dose and used on days 2 and 3.

On days 2 and 3 350 mg ZD2138 (Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK) or matching placebo was given after an overnight fast in a randomised, double blind fashion. Four hours later aspirin challenge was performed with the threshold dose as determined on day 1. Continuous ambulatory ECG was monitored on days 2 and 3 for 24 hours. Urine was collected before drug or placebo administration and then immediately before and three, six, and nine hours after aspirin challenge. Blood for determination of ex vivo LTB_4 generation was taken before and four (pre-aspirin), 12, and 24 hours after drug or placebo administration.

SPIROMETRY AND ASPIRIN CHALLENGE

Two recordings of FEV_1 were made with a dry wedge bellows spirometer (Vitalograph Ltd, Buckingham, UK) at each time point and the highest value was used. Aspirin was prepared in

gelatin capsules of 10, 30, and 100 mg by the Guy's Hospital pharmacy. On study day 1 screening for aspirin sensitivity by aspirin challenge was performed. The starting dose of aspirin was 30 mg, after which measurements of FEV_1 were made at 15 minute intervals for two hours. The challenge was stopped if the FEV_1 had fallen by 20% or more within this time. If the fall was less than 20% and the subject was within 10% of baseline FEV_1, further incremental doses of aspirin were given in the order 60, 100, 300, and 600 mg at two-hourly intervals. A fall in FEV_1 of between 10% and 20% was allowed to return to within 10% of baseline before the next dose was administered. The dose that elicited a fall in FEV_1 of 20% or more was considered the threshold dose. On study days 2 and 3 the threshold dose of aspirin was administered four hours after dosing with placebo or ZD 2138 and FEV_1 was measured at 15 minute intervals for two hours and then hourly until six hours after aspirin administration. All subjects were asked to report any new symptoms or change in symptoms after aspirin ingestion from the baseline period and these were recorded. Antihistamines were discontinued for 48 hours, inhaled and nasal corticosteroids for 24 hours, and salbutamol for at least eight hours before each study day until 24 hours after aspirin challenge.

MEASUREMENT OF URINARY LTE₄

The free radical scavenger 4-hydroxy-2,2,6,6-tetramethylpiperidinooxy free radical (4-hydroxy-TEMPO; Sigma Chemical Company, Poole, Dorset, UK) was added to each urine sample immediately after collection to a final concentration of 1 mmol/l, the samples were adjusted to pH 9 with NaOH to stabilise endogenous leukotriene metabolites and stored at −70°C until analysed. Urinary LTE_4 levels were measured as described previously.[18] The stored urine samples were thawed and 50 μl of a solution containing [³H] LTE_4 (128·5 Ci/mmol; NEN, DuPont, Boston, USA) was added to 10 ml urine to give 4000 disintegrations per minute. After adding 250 μl glacial acetic acid to bring the pH to 3·5–3·8 the 10 ml sample was loaded onto a 10 μm precolumn (Ultrasil ODS, 3·4 mm × 4·5 cm, Beckman, USA) to wash off polar metabolites with a phosphate buffer (0·1% NaH_2PO_4, pH 3·8) for eight minutes and then for 12 minutes with methanol:phosphate buffer in the proportions 50:50 (v:v). The sample was then retrogradely eluted onto a reversed phase analytical column (Ultrasil ODS 4·5 mm × 25 cm, Altex) that had been equilibrated in 62% methanol:37·8% water:0·1% acetic acid:0·1% EDTA (v:v:v:w) at pH 5·6 at a flow rate of 1 ml/min. One minute fractions were collected and the fraction containing peak radioactivity corresponding to the internal standard was identified by counting 250 μl of the eluent in a β liquid scintillation counter (Tricarb 1900CA, Packard Instrument Co, USA). The remaining 750 μl of those fractions containing [³H] LTE_4 and the two fractions eluted before and after the peak were dried under vacuum and resuspended in 250 μl of

Table 1   Baseline FEV_1 values, medication, and threshold dose of aspirin in the seven subjects

Subject	Age (years)	Sex	FEV_1 (litres)	% predicted FEV_1	Drugs	Aspirin dose (mg)
1	37	M	2·46	67	S, B	30
2	44	F	2·29	85	S	30
3	35	M	3·40	91	S, B	60
4	35	F	2·24	89	S, B, nB	40
5	48	M	3·04	82	S, B, nF	60
6	46	M	2·28	81	Bu, nB, T	100
7	52	F	2·29	105	Bu, Tn	60

S = salbutamol; B = beclomethasone dipropionate; Bu = budesonide; T = terfenadine; Tn = terbutaline; nB = nasal beclomethasone; nF = nasal fluticasone.

10 mmol/1 Tris HCl buffer (pH 7·4). The concentration of immunoreactive LTE_4 was assessed by radioimmunoassay as described previously.[19] Urinary creatinine concentrations were measured (Mira Analyser, Hoffman Roche, Welwyn) by the kinetic picrate method, without deproteinisation.[20] LTE_4 concentrations were expressed as pg/mg creatinine and were calculated from measured immunoreactivity minus background, corrected for recovery and dilution.

MEASUREMENT OF EX VIVO LTB_4

The ability of ZD2138 to inhibit 5-lipoxygenase was also assessed by measurement of calcium ionophore-stimulated ex vivo LTB_4 production in whole blood. Blood was collected into heparinised tubes and calcium ionophore (A23187) added to 500 μl whole blood in triplicate to a final concentration of 2·5 μmol/l. The blood was incubated for 20 minutes at 37°C, centrifuged at 3600 rpm for one minute, and the supernatant stored at −20°C. LTB_4 was assayed subsequently by radioimmunoassay, as previously described.[21]

DATA ANALYSIS

The area under the FEV_1–time curve following administration of placebo or ZD2138 was calculated for the four hours before aspirin challenge to assess bronchodilatation and for the six hours after aspirin challenge and analysed nonparametrically by the Wilcoxon signed rank test. Using linear regression analysis, the percentage inhibition of urinary LTE_4 was correlated with the degree of protection by ZD2138 against a fall in FEV_1 for each of the periods −4–0, 0–2, and 2–6 hours after aspirin ingestion. Baseline FEV_1 and urinary LTE_4 values were also correlated with the degree of bronchodilatation in each individual using linear regression analysis. The urinary LTE_4 values were expressed as pg/mg creatinine and the area under the urinary LTE_4–time curve for the drug and placebo days were also compared using the Wilcoxon signed rank test. Data for LTB_4 values were only available for five of the seven subjects because of technical difficulties with the other samples. All data points are expressed as mean (SE).

Results

SUBJECTS

On the screening day all subjects experienced symptoms of wheeze following aspirin ingestion and, in addition, subjects 1, 2, 3, 6, and 7 experienced rhinorrhoea and nasal congestion. Table 2 describes the symptoms experienced by the subjects on the study days. In all subjects ZD2138 premedication reduced aspirin-induced symptoms.

AIRWAY RESPONSES

The resting FEV_1 before drug administration was significantly lower on the ZD2138 day (2·36 l) than on the placebo day (2·71; p < 0·05). Figure 1 shows the mean change in FEV_1 for four hours after administration of placebo or ZD2138. FEV_1 rose by 7 (2·3)% at three hours after administration of ZD2138 compared with a fall of 4 (3·9)% after placebo (p < 0·05). The mean FEV_1 at four hours after drug/placebo administration and immediately before aspirin challenge was 2·43 (0·16) l after ZD2138 and 2·57 (0·17) l after placebo (p > 0·05). There was no correlation between the degree of bronchodilatation and baseline FEV_1, baseline urinary LTE_4, or asthma medication.

There was overall protection by ZD2138 of the fall in FEV_1 induced by aspirin challenge (p = 0·05) as shown in fig 2. On the placebo day

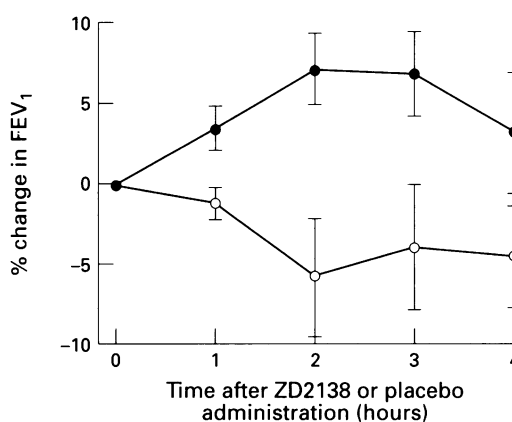*Figure 1   Percentage change in FEV_1 for the four hours after administration of ZD2138 (●) or placebo (○), and before aspirin challenge.*

Table 2   Symptoms related to aspirin ingestion after premedication with placebo or ZD2138

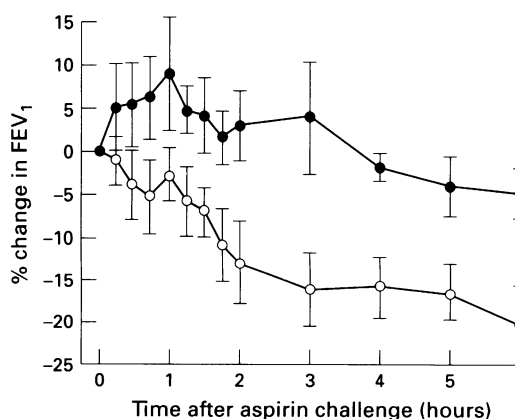| Subject	Placebo day	ZD2138 day
1	Rhinorrhoea, conjunctival streaming, wheeze	—
2	Wheeze, sneezing	Nasal tickle
3	Rhinorrhoea, epigastric discomfort, wheeze	—
4	Nausea, vomiting, headache, wheeze	Nausea, headache
5	Wheeze	—
6	Nasal congestion, severe fatigue, nausea, chest pain, wheeze	Rhinorrhoea, conjunctival streaming, tiredness
7	Wheeze, rhinorrhoea, sneezing, headache	—

Figure 2   Mean (SE) percentage change in FEV_1 for six hours after aspirin challenge. ZD2138 (●) or placebo (○) was administered four hours before aspirin challenge.

Table 3 Maximum change in FEV_1 for each of the seven subjects during the periods 0–2 and 2–6 hours

Subject	0–2 hours		2–6 hours		Evaluation	
	P	D	P	D	0–2 hours	2–6 hours
1	−10	0	−11	−8	R	NR
2	−12	+2	−25	−9	R	R
3	−36	+18	−47	+7	R	R
4	+5	−7	−15	−10	NE	PR
5	−22	0	−17	+2	R	R
6	−10	−9	−17	−19	NR	NR
7	−15	0	−18	−10	R	PR
Mean (SE)	−14 (5)	0·6 (3)	−21 (6)	−7 (3)		

Based on the fall in FEV_1 and the difference between drug (D) and placebo (P) days, each subject has been categorised as a responder (R), partial responder (PR), or non-responder (NR) to ZD2138. NE = non-evaluable.

there was a mean maximal fall in FEV_1 of 20·3 (4·9)% six hours after administration of aspirin compared with 4·9 (2·9)% on the ZD2138 day. Individual data are shown in fig 3. In subjects 3 and 5 there was complete inhibition and in subjects 1, 2, and 7 there was partial protection of the aspirin-induced asthmatic reaction. In subject 4 there was no inhibitory effect of the 5-lipoxygenase inhibitor on aspirin-provoked bronchoconstriction.

The effects of ZD2138 on aspirin-induced responses are similarly apparent if the maximal falls in FEV_1 in the first two hours and for the 2–6 hour period are analysed. In the first two hours five of the seven individuals clearly showed attenuation of the aspirin-induced response by active drug. Subject 4 could not be evaluated during the first two hours because she did not have a significant fall in FEV_1. Similarly, for the 2–6 hour period five of the seven individuals showed a response (table 3).

There was no significant correlation between inhibition of urinary LTE_4 levels and protection by ZD2138 against a fall in FEV_1 induced by aspirin for the periods −4–0 hours ($r = 0·37$; $p < 0·5$), 0–2 hours ($r = 0·43$; $p < 0·5$), and 2–6 hours ($r = 0·3$; $p = 0·5$) after aspirin ingestion.

INHIBITION OF EX VIVO GENERATION OF LTB_4
Figure 4 shows the mean ionophore-induced generation of LTB_4 from whole blood after administration of placebo and ZD2138. The baseline LTB_4 levels were 102·4 (30) µg/ml and 97·1 (26) µg/ml on the ZD2138 and placebo days, respectively. There was substantial inhibition of ex vivo generation of whole blood LTB_4; compared with placebo there was an average 57% inhibition at four hours, 72% inhibition at 12 hours, and 76% at 24 hours after dosing with ZD2138.

URINARY LTE_4
There was no significant difference between baseline urinary LTE_4 levels on the two days (fig 5). On the placebo day urinary LTE_4 levels did not change significantly from the baseline value of 391 (72) pg/mg creatinine to 319 (64) pg/mg creatinine at four hours after placebo administration immediately before aspirin challenge ($p < 0·05$). LTE_4 excretion increased to

806 (369) pg/mg creatinine at three hours, 1853 (986) pg/mg creatinine at six hours, and 1743 (482) pg/mg creatinine at nine hours after aspirin challenge. After administration of ZD2138 there was a fall in urinary LTE_4 levels in all seven subjects from a baseline value of 402 (100) pg/mg creatinine to 180 (47) pg/mg creatinine immediately before aspirin challenge ($p < 0·05$). The urinary LTE_4 concentration was 275 (181) pg/mg creatinine at three hours, 487 (405) pg/mg creatinine at six hours, and 713 (573) pg/mg creatinine at nine hours after aspirin challenge. Compared with placebo there was 43% inhibition at four hours, 70% at seven hours, 74% at 10 hours, and 59% at 13 hours after dosing with ZD2138. Significantly less urinary LTE_4 excretion was seen after ZD2138 than after administration of placebo ($p < 0·05$).

ZD2138 was well tolerated and no adverse events attributed to the drug occurred in the study. All seven subjects completed the study.

Discussion

The ability of ZD2138 to modulate aspirin-induced asthma has been investigated in seven asthmatic subjects with aspirin sensitivity. ZD2138 is a novel non-redox 5-lipoxygenase inhibitor which is derived from a unique enantioselective series. It is well tolerated and at single oral doses of 350 mg or more inhibits leukotriene synthesis for 24 hours.[17] The drug was given to fasted subjects to standardise bioavailability. The study was designed so that aspirin provocation was undertaken four hours after drug administration when the plasma level of the drug is anticipated to be maximal. Changes in pulmonary function were measured using FEV_1, and the effectiveness of 5-lipoxygenase inhibition was assessed by measuring LTB_4 generation of calcium ionophore-activated whole blood leucocytes and excretion of immunoreactive LTE_4 into the urine.

We have shown that 5-lipoxygenase inhibition by ZD2138 leads to significant bronchodilatation and protection against the fall in FEV_1 induced by oral aspirin challenge in individuals with aspirin-sensitive asthma. In addition, each patient experienced significantly fewer naso-ocular and/or systemic symptoms in response to aspirin after dosing with ZD2138 compared with placebo. The reduction in systemic symptoms was observed even in the individual who showed no amelioration of the decrement in FEV_1 following aspirin ingestion after premedication with the active drug (subject 4).

The heterogeneity of FEV_1 responses to the inhibitory effects of antileukotriene drugs has been reported previously.[14] This may reflect heterogeneity of mechanisms for aspirin-sensitive asthma, inadequate 5-lipoxygenase inhibition in some individuals, or involvement of other mediators in the bronchoconstriction produced by aspirin-sensitive asthma. In this regard, naso-ocular symptoms induced by either oral aspirin or inhaled lysine-aspirin are associated with histamine release into nasal lavage fluid.[22 23] Bosso *et al* recently reported that histamine and tryptase release in venous blood
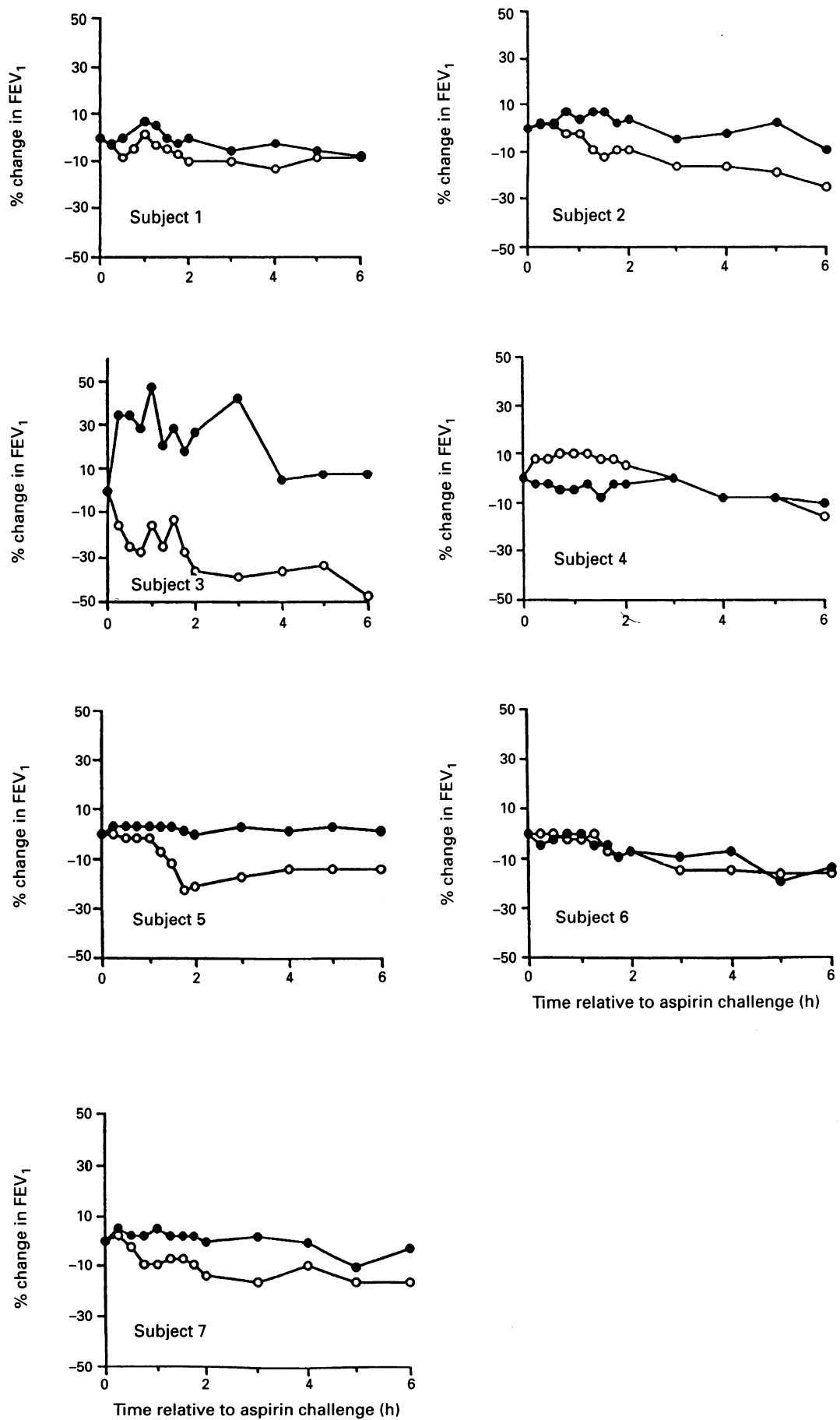
Figure 3 Individual graphs for all seven subjects demonstrating the percentage change in FEV_1 for six hours following aspirin challenge. ZD2138 (●) or placebo (○) was administered four hours before aspirin challenge.
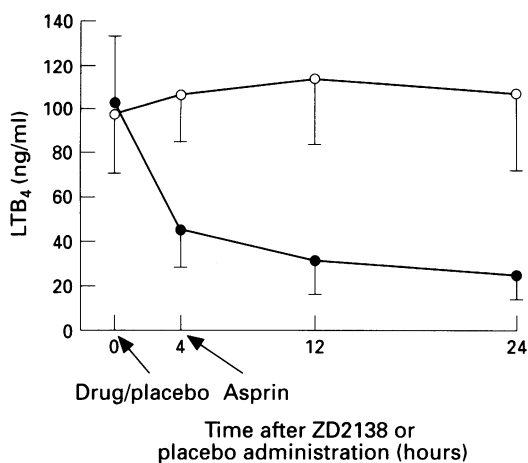
Figure 4   Mean (SE) whole blood ex vivo LTB₄ generation in response to 2·5 μmol/l A23187 for five of the seven subjects' before and after receiving ZD2138 (●) or placebo (○). Aspirin was administered immediately after the four hour time point. The data for two subjects are not shown because of technical difficulties in analysis of samples.
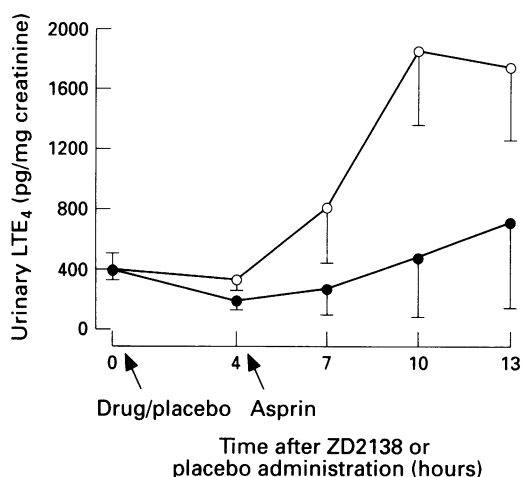

Figure 5   Mean (SE) urinary LTE₄ levels for the seven subjects before and after receiving ZD2138 (●) or placebo (○). Aspirin was administered four hours after dosing.

samples occurred in three out of four aspirin-sensitive asthmatic subjects following aspirin challenge. These three subjects experienced bronchospasm which was accompanied by gastrointestinal and/or skin reactions. There was no increase in tryptase in the remaining patients who developed bronchospasm but did not experience extrapulmonary symptoms.[24]

In all subjects ZD2138 inhibited the production of LTB₄ by whole blood leucocytes stimulated by calcium ionophore, and this inhibitory activity was present for 24 hours. There was a significant rise in urinary LTE₄ excretion in all patients following aspirin ingestion on the placebo day. ZD2138 completely attenuated the rise in urinary LTE₄ excretion in all but one subject (subject 6) in whom no difference was seen between the two study days. Thus, there may have been incomplete attenuation of

5-lipoxygenase, accounting for the relative ineffectiveness of the drug in attenuating the pulmonary response to aspirin-sensitive asthma in this individual. Ex vivo LTB₄ generation was inhibited by 51%, 38%, and 68% at four, 12, and 24 hours in this individual, however, thereby emphasising possible discordance in the different assays for demonstrating in vivo 5-lipoxygenase inhibition and their relevance to clinical efficacy. In subject 4 there was a substantial increase in urinary LTE₄ levels following ingestion of aspirin on the placebo day which was completely inhibited by ZD2138. These observations suggest that the lack of response to ZD2138 in this subject was unlikely to be due to inadequate 5-lipoxygenase inhibition. The possibility that the drug did not reach adequate concentrations locally in the airways cannot be excluded.

The increase in airway calibre following administration of ZD2138 is compatible with the reported effects of another 5-lipoxygenase inhibitor, Zileuton,[25] and leukotriene antagonists,[26 27] on increasing airway calibre in asthmatic subjects. However, the results of the present study are confounded by the fact that the baseline FEV_1 before drug administration was significantly lower than that on the placebo day. The data may therefore be a statistical artefact and simply represent a regression towards the mean. Further studies are needed to define whether there is any significant effect of the active drug on the calibre of resting airways. In a previous study of the effects of ZD2138 on allergen challenge, drug administration did not increase baseline FEV_1 values. The asthmatic subjects in that study, however, had milder asthma and their resting FEV_1 was almost normal.[28]

The time-dependent decreases in FEV_1 following aspirin ingestion on the placebo day in this study were more prolonged than those reported previously. A notable difference between the present study and the earlier reports is that aspirin was administered just before food ingestion in the present investigation. It is therefore possible that food delayed aspirin absorption and the subsequent decrease in FEV_1. Future studies should consider using inhaled lysine-aspirin challenge to provoke asthma in aspirin-sensitive individuals to avoid interactions with food.

The mechanism of aspirin-sensitive asthma is incompletely understood. Although there is considerable evidence to suggest that cyclooxygenase inhibition increases LTC_4 synthesis in aspirin-sensitive asthmatic subjects, there is little evidence to suggest that this occurs as a result of diversion from the cyclooxygenase pathway to the 5-lipoxygenase at the relatively low doses of aspirin required to induce the response. The finding that pretreatment of sensitised human airways in vitro with indomethacin results in increased generation of LTC_4 following IgE-dependent challenge supports the view that cyclooxygenase inhibition augments increased leukotriene release.[29] Similarly, in the guinea pig model of antigen-induced anaphylaxis, pretreatment of animals with indomethacin results in an enhancement of

the pulmonary mechanical response to intravenous antigen which is accompanied by an increased generation of LTB_4.[30] Studies in humans with aspirin sensitivity also indicate that increased generation of leukotrienes may contribute to the mechanisms of aspirin-sensitive asthma. Ferreri *et al* found that, in patients who developed naso-ocular symptoms following ingestion of aspirin, concentrations of both LTC_4 and histamine from nasal lavage fluid increased significantly from baseline values after provocation.[22] There was no decrease in PGE_2 after administration of the low doses of aspirin required to induce the response. Intranasal challenge with lysine-aspirin also led to increased nasal concentrations of histamine and LTC_4 in aspirin-sensitive subjects, but not in aspirin-tolerant individuals.[23] Urinary concentrations of LTE_4 have been used to reflect systemic synthesis of LTC_4 following both oral challenge with aspirin and bronchial challenge with inhaled lysine-aspirin. Christie *et al* reported that ingestion of aspirin which produced a > 15% fall in FEV_1 was associated with a mean fourfold increase in urinary LTE_4. Placebo challenge for the same individuals and aspirin challenge in asthmatic subjects tolerant of aspirin were not associated with a change in FEV_1 or increments in urinary LTE_4 concentration.[12] These observations were confirmed in subsequent reports.[31 32] Christie *et al*[33] and Dahlen *et al*[34] have also reported significant rises in urinary LTE_4 following bronchial challenge with lysine-aspirin.

In contrast to the present study ZD2138 did not protect against allergen-induced asthma.[28] The designs of the allergen and aspirin challenge studies were identical and the dose of ZD2138 used in both studies was the same. This suggests that the mechanisms for allergen- and aspirin-induced bronchoconstrictions are different. Aspirin-induced asthma appears to be more dependent on 5-lipoxygenase pathway metabolites. This is supported by our previous finding that asthmatic patients with aspirin sensitivity have higher basal levels of LTE_4 excretion in the urine than asthmatic patients without aspirin sensitivity,[12 18] and the observation that subjects with aspirin-sensitive asthma are considerably more sensitive to the bronchoconstrictor effects of inhaled LTE_4 than aspirin-tolerant asthmatic subjects.[13] Aspirin-induced asthma may therefore be a more pertinent in vivo model for assessing the preliminary therapeutic efficacy of this class of novel drug than allergen challenge. Our findings emphasise the importance of choosing the correct model when testing the efficacy of new therapeutic agents in man.

MK886, a FLAP inhibitor, has been shown to inhibit allergen-induced bronchospasm.[35] Neither ZD2138[28] nor Zileuton[36] inhibited allergen-induced bronchospasm, but both protected against aspirin-induced falls in FEV_1.[37] It remains to be evaluated whether different classes of 5-lipoxygenase inhibitors exhibit heterogeneity in clinical efficacy. The present study is consistent with a significant role for leukotrienes in the mechanisms contributing to aspirin-sensitive asthma, since inhibition of 5-

lipoxygenase by ZD2138 led to a substantial attenuation of the pulmonary response to aspirin in these patients.

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