Participation of the cysteinyl leukotrienes in the acute bronchoconstrictor response to inhaled platelet activating factor in man

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Abstract

To determine whether the effects of platelet activating factor on the airways may be due to the production of leukotrienes we studied the effects of pretreatment with the selective cysteinyl leukotriene antagonist SK&F 104353-Z_2 on the airway and cellular responses to inhaled platelet activating factor. Eight healthy men were studied in a randomised, double blind placebo controlled crossover study. A single dose of platelet activating factor that caused a fall of at least 35% in specific airways conductance (sGaw) was determined initially for each subject. Challenge with this dose of platelet activating factor was then carried out on two further occasions after pretreatment with a single nebulised dose of SK&F 104353-Z_2 or placebo. The % reductions in specific airways conductance and of partial flow at 30% of vital capacity ($P\dot{V}max_{30}$) were less after SK&F 104353-Z_2 than after placebo (22 versus 34 for sGaw, 19 versus 31 for $P\dot{V}max_{30}$). The mean (95% confidence limits (CL)) differences in the maximum % fall from control values for SK&F 104353-Z_2 and placebo were -12.6 (-23.8, -1.4) for sGaw and -12.5, (-20.8 -4.2) for $P\dot{V}max_{30}$. The mean % fall in neutrophil count was similar after SK&F 104353-Z_2 (46%) and after placebo (50%) (95% CL of difference 13.6, 6.6). Bronchial responsiveness to methacholine did not increase above baseline values in any subject when measured two weeks after challenge by platelet activating factor. This study suggests that leukotrienes play a part in the response to platelet activating factor in man.

Asthma has an important inflammatory component, in which numerous mediators may play a part.[1] Complex interactions between mediators have been reported,[2-5] and these, by amplifying the effects of individual mediators, could cause bronchial responsiveness to be increased.[1]

The cysteinyl leukotrienes and platelet activating factor (PAF) cause acute bronchoconstriction and increased bronchial responsiveness in various animals, and may be important mediators in asthma.[6-8] Inhaled PAF causes acute bronchoconstriction in man, a transient fall in circulating neutrophils,

flushing, and a desire to cough.[9 10] Leukotriene D_4 is one of the most potent naturally occurring bronchoconstrictors known, being up to 6000 times more potent than histamine.[11] A prolonged increase in bronchial responsiveness has been reported to follow inhalation of PAF in normal subjects[9] and of leukotriene E_4 in asthmatic subjects.[12]

The effects of PAF may depend on secondary leukotriene production. Human eosinophils produce large amounts of leukotriene C_4 when stimulated by PAF,[13] and PAF induced mucus production in human airways is reduced by a 5-lipoxygenase inhibitor in vitro.[14] The administration of PAF results in leukotriene formation[15] or biological effects that are reduced by 5-lipoxygenase inhibitors and leukotriene receptor antagonists in some animal models,[16-18] though these findings have not been confirmed in other studies.[19 20] Interpretation of the animal work is made difficult by considerable interspecies variation in the effects of lipid mediators, and by the lack of potency and selectivity of the earlier leukotriene antagonists and enzyme inhibitors.

SK&F 104353-Z_2 (2-(S)-hydroxy-3(R)-[(2-carboxyethyl)thio]-3-[2-(8-phenyloctyl)phenyl]-propanoic acid), a structural analogue of leukotriene D_4, is a potent and selective antagonist of the cysteinyl leukotrienes.[21] It inhibits the bronchoconstriction induced by leukotriene D_4, but not histamine, in man[22] and is a potent antagonist of LTC_4 and LTE_4 induced contraction of human bronchus in vitro.[23] It has no effect on PAF or histamine induced contraction of guinea pig trachea, or phosphodiesterase activity in canine tracheal smooth muscle.[23] We have studied the effects of inhaled SK&F 104353-Z_2 on the responses to inhaled PAF in man.

Methods

SUBJECTS

Eight non-smoking healthy men (aged 22–32 years) gave signed informed consent to participate in the study, which was approved by the hospital ethics committee. No subject gave a history of asthma and all had bronchial responsiveness to methacholine above 8 mg/ml.[24] Two subjects had positive skinprick test responses to common allergens; one, with a history of mild hay fever, was studied outside the pollen season. No subject was taking any medication and beverages containing caffeine were not consumed for at least six hours before any challenge. The results of physical

examination, chest radiography, electrocardiography, and routine haematological and biochemical screening were all within normal limits.

MATERIALS

Methacholine hydrochloride (Sigma Chemical Company, St Louis, Missouri) was made up by the hospital pharmacy in doubling concentrations from 0·5 to 64 mg/ml. C-18 PAF (Bachem UK Ltd, Saffron Waldron, Essex) was obtained as a dry powder, divided into 2·5 mg aliquots in ethanol, and stored in polypropylene tubes at $-80°C$. Immediately before PAF challenge, the ethanolic PAF was dried with a stream of nitrogen and resuspended in 0·9% saline with 2·5 mg/ml human serum albumin (Immuno Ltd, Sevenoaks, Kent) to produce a concentration of 800 mg/ ml. SK&F 104353-Z_2 and matched placebo were provided as clear phosphate buffered solutions at pH 7·4 for nebulisation.

METHACHOLINE CHALLENGE

Methacholine challenge was performed according to a modified Cockcroft protocol.[24][31] Bronchial responsiveness was determined as the provocative concentration of methacholine causing a 35% fall in specific airways conductance ($PC_{35}sGaw$) and also as the concentration causing a 30% fall in flow at 30% above residual volume from a partial expiratory flow-volume curve[25] ($PC_{30}P\dot{V}max_{30}$). Measurements of sGaw were obtained by panting manoeuvres in an automated pressure-flow plethysmograph[26] (Gould 2800 autobox, Cardiokinetics Ltd, Salford) and of $P\dot{V}max_{30}$ with a rolling seal spirometer (PK Morgan, Gillingham, Kent) attached to a differentiator (PK Morgan) and X–Y recorder (Rikadenki Mitsui Electronics UK Ltd, Chessington, Surrey). Diluent (0·9% saline with 0·5% chlorbutol BP) was inhaled by tidal breathing from a Wright nebuliser (output 0·13 ml/min) for two minutes, and measurements of sGaw (mean of eight values) and $P\dot{V}max_{30}$ (mean of two values) were obtained over the next five minutes. Doubling concentrations of methacholine were inhaled at 10 minute intervals in the same manner until the endpoint of the challenge was reached or the highest concentration of methacholine had been given. The endpoint of the challenge was defined as a fall in sGaw of at least 35% below the post-diluent value. The results were expressed as a percentage of post-diluent values. $PC_{35}sGaw$ and $PC_{30}P\dot{V}max_{30}$ were determined by linear interpolation from log_{10} dose-response curves. When $P\dot{V}max_{30}$ fell by less than 30%, $PC_{30}P\dot{V}max_{30}$ was obtained by extrapolation provided that a definite trend in the dose-response curve was apparent.

PAF CHALLENGE

PAF challenges were performed at a similar time of day for each subject. Baseline measurements of forced expiratory volume in one second (FEV_1, mean of three values) were obtained with a dry wedge bellows spirometer (Vitalograph Ltd, Buckinghamshire), followed by measurements of sGaw (mean of 16 values) and of $P\dot{V}max_{30}$ (mean of five values). The subject took 10 inhalations of diluent (human serum albumin 2·5 mg/ml in 0·9% saline) from a Microneb III nebuliser (Lifecare Hospital Supplies Ltd, Market Harborough, Leicester) attached to a Nebi-check dosimeter (PK Morgan) by tidal breathing while wearing a nose clip. Control measurements of sGaw were made at one minute and of $P\dot{V}max_{30}$ (mean of five values) at three minutes; venous blood was obtained for a differential white cell count at five minutes. The dosimeter was driven by air at 204 kPa (30lb/in^2) with a flow to the nebuliser of 8 l/ min. The volume median diameter of the aerosol particles was 4–5 μm, and gravimetric assessment showed the nebuliser output to be 6·4 × 10^{-3} ml per actuation,[31] giving a predicted dose of PAF of 9·6 × 10^{-8} mol per 10 inhalations.

Two millilitres of the PAF solution was placed in the nebuliser reservoir and 10 inhalations were taken as described for diluent. The ECG was monitored continuously and blood pressure measured (automatic sphygmomanometer) on completion of the inhalation. Measurements of sGaw (mean of eight values) were made at one and six minutes, and of $P\dot{V}max_{30}$ (mean of two values) at three and eight minutes. Venous blood was obtained for a white cell count at five minutes. If the maximum mean fall in sGaw was less than 35% of the control value, the challenge was repeated two weeks later with 20 inhalations of the same concentration of PAF. Subjects were excluded from further study if bronchoconstriction did not occur at the higher dose because of the potential adverse cardiovascular effects of large doses of PAF.

PROTOCOL

Bronchial responsiveness to methacholine was assessed initially (baseline), and repeated two weeks after each PAF challenges. After the bronchoconstricting dose of PAF had been determined the PAF challenge was repeated on two treatment days, at least two weeks apart, after prior inhalation of either SK&F 104353-Z_2 or placebo in a randomised, double blind, crossover study design. The methacholine challenge test was at least 24 hours before challenge with PAF, placebo, or SK&F 104353-Z_2.

TREATMENT DAYS

Baseline pulmonary function was measured as for the PAF challenge. Three millilitres of 400 μg/ml SK&F 104353-Z_2 or placebo was placed in the reservoir of a "Pulmo-Sonic" ultrasonic nebuliser (DeVilbiss Company, Somerset, Pennsylvania). The subject inhaled the aerosol by tidal breathing, wearing a nose clip, for 10 minutes and pulmonary function measurements were repeated. Drug delivery was estimated volumetrically. PAF challenge was performed 15 minutes after the inhalation was completed with the bronchoconstricting dose of PAF previously determined for each subject.

STATISTICAL ANALYSIS

When a $PC_{30}P\dot{V}max_{30}$ could not be extrapolated (eight out of 32 methacholine challenges) the points were entered into the analysis of variance as missing data (Minitab software handbook).

The coefficient of variation (SD as a percentage of the mean) for each subject was calculated for baseline values of sGaw, $P\dot{V}max_{30}$, and FEV_1 obtained before each methacholine and PAF challenge, and at the beginning of each treatment day. The lowest mean sGaw and $P\dot{V}max_{30}$ values after inhalation of PAF and the circulating neutrophil count at five minutes were expressed as percentages of the control post-diluent values. The effect of SK&F 104353-Z_2 and placebo on pulmonary function was assessed by comparing the changes in FEV_1, sGaw, and $P\dot{V}max_{30}$ after treatment by paired t tests. Mean differences and 95% confidence limits (95% CL) for the mean differences are given.

The reproducibility of the PAF challenge was assessed by comparing the greatest mean change in sGaw, $P\dot{V}max_{30}$, and neutrophil count after PAF challenges on dose finding and placebo treatment days, and expressed as the mean difference and coefficient of repeatability for each measurement. The coefficient of repeatability was calculated from the formula

$$2x\sqrt{\frac{\sum(D_1 - D_2)^2}{n}},$$

where D_1 and D_2 are the maximum mean reductions obtained after the first and second challenge and n is the number of subjects. This defines 95% of the differences between the two challenges, and is the coefficient of repeatability adopted by the British Standards Institution.[27] PC values were log transformed for the analysis. Changes in PC values two weeks after each PAF challenge were compared with baseline values by paired t tests and by a one way analysis of variance. All data were analysed by means of the Minitab statistical package (Minitab Inc, State College, Pennsylvania).[28]
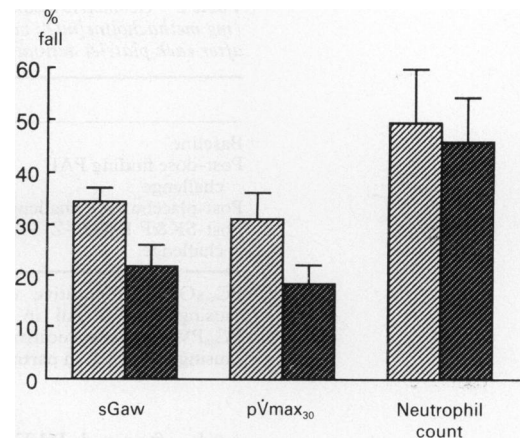
Results

BASELINE PULMONARY FUNCTION

The mean (range) coefficients of variation for baseline values were: FEV_1 3·76% (2·3–5·8%), sGaw 9·6% (4·3–14·4%), and 8·9% (6·0–20·3%) for $P\dot{V}max_{30}$. These are similar to previously published values.[29][30]

PAF CHALLENGE AND TREATMENT DAYS

PAF challenge was well tolerated with no

Mean PAF induced falls in specific airways conductance (sGaw), partial flow at 30% of vital capacity ($P\dot{V}max_{30}$), and circulating neutrophil count after inhalation of SK&F 104353-Z_2 (▨) and placebo (■). Bars represent standard errors of the mean. The y axis represents percentage fall from control values obtained after the inhalation of diluent.

serious side effects. Four subjects needed 10 inhalations of PAF and four 20 inhalations to produce the required fall in sGaw. All subjects experienced transient irritation of the throat, facial flushing, and a desire to cough. The maximum mean fall in sGaw and $P\dot{V}max_{30}$ always occurred one and three minutes respectively after PAF inhalation. No changes in heart rate, blood pressure, or cardiac rhythm were observed.

No symptoms were reported by any subject after inhalation of either SK&F 104353-Z_2 or placebo. The mean volume of solution nebulised was 1·7 ml (SD 0·40, range 1·2–2·6 ml), equivalent to 680 (range 480–1040) μg of SK&F 104353-Z_2. There were no significant changes in pulmonary function after SK&F 104353-Z_2 or placebo (table 1).

There was a fall in sGaw, $P\dot{V}max_{30}$, and circulating neutrophil count after PAF challenges on the two treatment days (figure). The maximum fall after SK&F 104353-Z_2 was less than that seen after placebo for sGaw (22% v 34%) and $P\dot{V}max_{30}$ (19% v 31%). The mean % difference in the fall in sGaw on the two treatment days was 12·6 (95% CL 23·8, 1·4), $p < 0·05$, and for $P\dot{V}max_{30}$ was 12·5 (95% CL 20·8, 4·2), $p < 0·01$. The mean difference in the PAF induced % fall in neutrophil count between SK&F 104353-Z_2 and placebo was 3·5 (46·1 v 49·6, 95% CL −13·6, 6·6), $p > 0·1$.

PAF challenge resulted in similar reductions in sGaw, $P\dot{V}max_{30}$, and circulating neutrophil count on dose finding and placebo treatment days (mean difference (coefficient of repeatability) in the maximum % fall on the two days: sGaw 6 (25), $P\dot{V}max_{30}$ 3 (36), neutrophil count 9% (33)).

METHACHOLINE RESPONSIVENESS

Bronchial responsiveness to methacholine two weeks after each PAF challenge did not change by more than one doubling concentration of methacholine from baseline values in any subject. There was a trend for $PC_{35}sGaw$ and PC_{30} $P\dot{V}max_{30}$ methacholine to be higher two

Table 1   Mean (SD) values of FEV_1, partial flow at 30% of vital capacity ($P\dot{V}max_{30}$), and specific airways conductance (sGaw) before and immediately after inhalation of SK&F 104353-Z_2 or placebo

	Pretreatment		Post-treatment	
	SK&F 104353-Z_2	Placebo	SK&F 104353-Z_2	Placebo
FEV_1 (l)	4·54 (0·75)	4·44 (0·69)	4·46 (0·71)	4·42 (0·70)
$P\dot{V}max_{30}$ (l s⁻¹)	3·22 (0·85)	3·30 (0·84)	3·06 (0·92)	3·09 (0·91)
sGaw (s⁻¹ kPa)	3·40 (0·50)	3·26 (0·65)	3·48 (0·53)	3·59 (0·78)

Table 2 Geometric mean $PC_{35}sGaw$ and $PC_{30}P\dot{V}max_{30}$ (mg methacholine/ml): values at baseline and two weeks after each platelet activating factor (PAF) challenge

	$PC_{35}sGaw$	$PC_{30}P\dot{V}max_{30}$
Baseline	7·2	7·5
Post-dose finding PAF challenge	10·2	12·2
Post-placebo PAF challenge	12·7	8·6
Post-SK&F 104353-Z$_2$ PAF challenge	14·1	13·4

$PC_{35}sGaw$—provocative concentration of methacholine causing a 35% fall in specific airways conductance; $PC_{30}P\dot{V}max_{30}$—provocative concentration of methacholine causing a 30% fall in partial flow at 30% of vital capacity.

weeks after each PAF challenge than at baseline (table 2), but this was not significant (one way analysis of variance: for $PC_{35}sGaw$ F = 1·25, p = 0·31, and for $PC_{30}P\dot{V}max_{30}$ F = 0·55, p = 0·65).

Discussion

In this study in normal subjects treatment with SK&F 104353-Z$_2$, a structural analogue of leukotriene D$_4$, reduced the C-18 PAF induced falls in both sGaw and P$\dot{V}$max$_{30}$ by 30%. The effect of SK&F 104353-Z$_2$ on the response to PAF has not been investigated in human tissue in vitro, but the compound has no activity as a PAF antagonist in animal studies,[23] and appears to be an extremely selective cysteinyl leukotriene receptor antagonist.[22 23] Our findings suggest therefore that at least a proportion of PAF induced bronchoconstriction in man is dependent on the secondary production of cysteinyl leukotrienes. The absence of any reduction in the PAF induced fall in circulating neutrophil count suggests that the effects of PAF on circulating neutrophils occurs through a different mechanism.

The single dose PAF challenge used in this study was well tolerated, and resulted in more reproducible acute changes in pulmonary function and circulating neutrophil count than did a previous cumulative dose-response challenge.[31] This may be because a single dose challenge avoids the problem of tachyphylaxis encountered with repeated doses of PAF over a short period.[9 31]

Treatment with 800 μg of SK&F 104353-Z$_2$ 15 minutes before a single inhalation of leukotriene D$_4$ reduced the mean fall in sGaw by 75% (from 55% to 14·3%) in normal subjects.[21] Our results suggest that SK&F 104353-Z$_2$ also antagonises the effects of endogenous cysteinyl leukotriene production in man. Whether SK&F 104353-Z$_2$ is an equipotent antagonist of endogenous cysteinyl-leukotrienes and exogenous inhaled leukotrienes is unknown, so we are unable to estimate the proportion of PAF induced bronchoconstriction that is related to secondary cysteinyl leukotriene production.

The transient fall in circulating neutrophils observed after inhaled PAF is due to temporary sequestration of these cells within the pulmonary circulation.[32] Cysteinyl leukotrienes have no known chemotactic properties in man, so it

is not surprising that pretreatment with SK&F 104353-Z$_2$ did not affect this response. This effect could, however, be due to secondary generation of leukotriene B$_4$, a potent chemotactic agent for the human neutrophil and known to be produced in close association with PAF.[33] Alternatively, this phenomenon could be due to a direct effect of PAF, mediated via specific PAF receptors on human neutrophils.[34]

This study did not examine the possibility that bronchial responsiveness was increased at an earlier time after inhaled PAF because, in a previous study,[31] we were unable to find any significant increase in bronchial responsiveness at any time after PAF inhalation. Bronchial responsiveness was measured two weeks after each PAF challenge in the present study to ensure that baseline responsiveness was unchanged before the next treatment day. No changes in bronchial responsiveness were found at this time, in agreement with our previous findings and those of other workers, who had used various doses of PAF.[35–37] The doses of PAF used in our study (9·6 $\times$ 10^{-8} mol or 1·9 $\times$ 10^{-7} mol) are similar to the geometric mean dose (1·1 $\times$ 10^{-7} mol) used in the study in which increased bronchial responsiveness occurred for up to four weeks after PAF inhalation in normal subjects.[9] There must now be some doubt that such a prolonged increase in bronchial responsiveness is a reproducible consequence of PAF inhalation in normal subjects.

The mode of action of PAF in man has not been completely determined. Indirect mechanisms may be important, as shown by the finding that PAF induced contraction of human bronchial smooth muscle is platelet dependent in vitro,[38] and that pretreatment with an antihistamine reduces PAF induced bronchospasm in vivo.[39] High affinity binding sites, which may represent specific PAF receptors, have been described in human lung homogenates.[40] Binding of PAF to these receptors may trigger complex intracellular transduction mechanisms that include activation of G proteins and protein kinase C, increased turnover of inositol phosphates, and a rise in intracellular Ca^{2+}. Phospholipase A$_2$ may then be activated, releasing arachidonate from membrane phospholipids with the subsequent production of a cascade of mediators, including leukotrienes, prostaglandins, and thromboxane.[41] The relative contributions of these different mediators in producing the biological effects attributed to PAF have yet to be determined.

Whether the metabolic pathways mediating interactions between PAF and leukotrienes are similar in normal and asthmatic subjects has not been determined, and any extrapolations from our study of inhaled PAF in normal subjects to the presumed effects of endogenous PAF in asthma must be cautious. Potent and selective PAF antagonists, 5-lipoxygenase inhibitors, and leukotriene receptor antagonists are becoming available for clinical trials; studies with these compounds will help to determine the relative importance of PAF and leukotrienes, and of interactions between them, in the pathophysiology of asthma.

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