Effect of selective phosphodiesterase 3 inhibition on the early and late asthmatic responses to inhaled allergen

Philip G. Bardin,¹ Martina A. Dorward, Fiona C. Lampe, Brigitte Franke² & Stephen T. Holgate

Immunopharmacology Group, University Medicine, Southampton General Hospital, Southampton, UK, ¹Internal Medicine, Stellenbosch University Pulmonary Unit for Research (SUPUR), University of Stellenbosch, Cape Town, SA, and ²Sandoz Pharma Limited, Basle, Switzerland

Aims Phosphodiesterase isoenzymes may play an important role in the regulation of airway calibre and bronchial smooth muscle function. Immunomodulatory actions may also be important in allergic airway inflammation. We have examined the effect of a phosphodiesterase (PDE) 3 inhibitor (MKS492) on the early and late responses to inhaled allergen in 18 atopic asthmatic subjects.

Methods On three separate occasions, 2–4 weeks apart, patients were administered either placebo, 20 mg or 40 mg of oral MKS492 as a single dose. After 90 min they inhaled a dose of allergen (grass pollen or *D. pteronyssinus*) that had been demonstrated to produce a 20% decrease in FEV₁. Measurements of FEV₁ were repeated at 30 min intervals over the subsequent 7.5 h.

Results After placebo, allergen inhalation produced bronchoconstriction with a maximum mean minimum FEV₁ of 75.9% (12.7) (mean [s.d.]) of post-saline baseline values and a late response developed in 13 subjects (decrease in FEV₁ ≥ 15%). MKS492 (40 mg) increased the mean minimum % post-saline FEV₁ at 30 min from 81.6% (14.0) for placebo to 93.1% (13.8), difference 11.5, 95% CI (2.6, 20.4). The mean minimum % post-saline FEV₁ in the early response (0–2 h) was also increased by the 40 mg dose to 86.5% (11.8) *vs* 77.9% (13.8) for placebo, but not by the 20 mg dose. Significant bronchodilatation was noted 90 min after dosing (pre-allergen) in the MKS492 40 mg group. Late phase responses as assessed by 4 h % post-saline FEV₁ was significantly improved by MKS 492 40 mg to 90.0% (6.3) *vs* 83.0% (12.4) for placebo—difference 7.0, 95% CI (0.1, 13.9), but again not by the 20 mg dose. Analysis of the area under the FEV₁ response-time course curves showed non-significant reduction in the late response after the 40 mg dose (placebo 159.0, 134.8) *vs* 128.1, [81.5] for 40 mg).

Conclusions A novel PDE 3 inhibitor significantly decreases the early bronchoconstrictor response in asthma and attenuates the late response; effects that may be more marked at higher doses.

Keywords: asthma, phosphodiesterase inhibitors, allergen

Introduction

Cyclic nucleotide phosphodiesterases (PDEs) are a family of cellular enzymes which inactivate the naturally occurring second messenger cyclic nucleotides adenosine 3',5'-monophosphate (cAMP) and guanosine 3',5'-monophosphate (cGMP). Seven isoenzyme families (PDE1–7) have now been characterised [1] and they control the intracellular concentration of these two cyclic nucleotides that play an important role in regulating airway function. Increased intracellular cAMP or cGMP relaxes airway smooth muscle after activation of adenylyl or guanylyl cyclase enzymes induced by receptor stimulation (β -adrenoceptor agonists, prostaglandin E, atrial natriuretic peptide) or directly as with nitric oxide. PDE1, 2, 3, 4 and 5 activity has been identified in human airway smooth muscle cells [2]. Cyclic nucleotides are also functionally

active in other cells of the airways and this may be of particular importance in the function of inflammatory cells. Recent evidence suggests that PDE 3/4 inhibitors may decrease the synthesis of cytokines in an animal model of asthma [3], and isoenzyme-specific inhibitory compounds may therefore have prominent immunomodulatory actions in allergic inflammation and asthma.

MKS492, 8-amino-3,7 dihydro-7-(2-methoxyethyl)-1,3 dimethyl-1H-purine-2,6-dione, is a PDE 3 selective xanthine analogue that has bronchodilator and anti-inflammatory properties. It depresses the spontaneous tone of human bronchial smooth muscle *in vitro* (Morley, unpublished data), reverses bronchoconstriction in the airways of anaesthetised mechanically-ventilated guinea-pigs (Morley, unpublished data) and is 40 times more potent than aminophylline in reversing sustained bronchospasm induced by bombesin [4]. Immunomodulatory effects were found in studies of allergen-induced accumulation of macrophages, eosinophils and neutrophils in guinea-pig airways which was inhibited by MKS492 administered by inhalation (Morley, unpublished

Correspondence: Professor P. G. Bardin, Department of Internal Medicine, University of Stellenbosch, PO Box 19063, Tygerberg 7505, Cape Town, SA.

data). Finally, the compound was able to attenuate the development of bronchial hyperresponsiveness to immune complexes and platelet activating factor (PAF) in the same model.

Recently, the potential value of selective PDE inhibition in the treatment of asthma has been reconsidered [5]. Selective compounds may exhibit bronchodilating as well as anti-inflammatory actions, and therefore inhibit both the early bronchoconstrictive phase of asthma as well as late phase inflammatory events. We evaluated the effect of MKS492 on both the early and late phases of the asthmatic reaction.

Methods

Patients

Twenty-one subjects entered the study. Three patients withdrew during the study because of exacerbations of their asthma and consequent changes in their medication. Eighteen atopic asthmatics (12 male), mean age (range) 34.7(18–56); FEV₁ (% predicted; mean [s.d.]) 89.1 (13.1) took part in the study (Table 1). Asthma was controlled by inhaled β_2 -adrenoceptor agonists and six patients also used inhaled corticosteroids. Subjects gave their written informed consent, and the study was approved by the Southampton University and Hospitals Ethical Sub-Committee.

Allergen bronchoprovocation

Before each of the study days, β_2 -adrenoceptor agonists and inhaled corticosteroids were withheld for 24 h and 7 days respectively. Patients attended the laboratory on 3 days at least 2 weeks, but not more than 4 weeks, apart. After 10 min of rest, FEV₁ was measured on three occasions 1 min apart, using a dry-wedge spirometer (Vitalograph Ltd,

Buckingham, UK), and the highest value recorded was used in subsequent analyses. Patients received either oral MKS492 40 mg, 20 mg or matched placebo as a single dose administered double-blind and in random order, and FEV₁ measurements were recorded 90 min later. They then inhaled 5 breaths of nebulised saline delivered by an Inspiron nebuliser (CR Bard International Ltd, Sunderland, UK) from a starting volume of 3 ml. The patients were instructed to take five breaths from end tidal volume to maximum inspiratory capacity.

Measurements of FEV₁ were again made 3 min after inhalation of saline. If FEV₁ had not fallen by >10% of baseline, allergen challenge was carried out (grass pollen: $n=13$, *D. pteronyssinus*: $n=5$, Bencard, Brentford, UK), with a fixed amount that, in a previous dose-response experiment, had been demonstrated to provoke a fall in FEV₁ of 20% from baseline. For each subject the allergen chosen for the allergen challenge was that producing the largest wheal response on skin testing. After allergen inhalation, measurements of FEV₁ were done every 10 min for the first hour and thereafter half-hourly for 7.5 h. Late phase responses were defined as a decrease in FEV₁ $\geq 15\%$ from post-saline baseline FEV₁ 2–7.5 h after allergen challenge. Timing of the challenges was standardized between visits.

Statistical methods

The trial was designed (for reasons of safety), that no patient received 40 mg of the active treatment until they had already received 20 mg on a previous trial day. Patients were randomized to one of four treatment combinations, 20 Placebo 40, Placebo 20 40, 20 Placebo 20 and Placebo 20 20, with group sizes of 6, 6, 3 and 3 respectively. Summary measures of each individual's FEV₁ response over time were used as the outcome variables for analysis.

Table 1 Baseline characteristics of patients.

	Age (years)	Sex	Baseline FEV ₁ (l)	FEV ₁ % predicted	Treatment
1*	40	F	2.3	90	S
2	19	M	4.1	99	S
3	30	M	3.6	90	S
4	39	M	2.7	87	S
5*	41	F	3.1	100	S
6	26	M	5.1	106	S
7*	56	F	2.25	100	S
8	18	M	3.8	94	S,B
9	38	M	3.8	95	S,B
10*	20	F	3.1	99	S
11	34	M	1.8	55	S,B
12	21	M	1.8	99	S,B,T
13	21	M	4.5	102	S
14*	26	F	2.0	95	S,B
15	55	M	3.0	85	S
16	30	M	2.8	76	S,A
17	29	F	2.7	77	S
18	27	M	2.9	70	S,B
Mean (s.d.)	32		3.2 (0.92)	90 (13.1)	

S, Salbutamol; B, Beclomethasone dipropionate; A, Antihistamine; T, Theophylline.

*Patients who developed only an early bronchoconstrictor reaction.

Comparisons between placebo and 20 mg active treatment were made using the two-treatment, two-period crossover design for all patients on their first and second trial visit. A standard analysis showed no evidence of treatment carry-over or of a period effect. Therefore, comparisons of 40 mg active treatment with 20 mg and with placebo were made using the paired data available for all 12 subjects who had received 40 mg on their final visit. A two-way analysis of variance was used with subjects and treatments as factors. If the overall *F* test for treatments was significant, pairwise comparisons were made using the pooled estimate of the within-subject variability from the analysis of variance. All analysis was carried out in SAS v6.04.

Results

Patients

Of 21 patients who had entered the study, 18 completed it. The baseline patient characteristics are shown in Table 1. Significant differences in FEV₁ were noted between baseline values obtained before and 90 min after receiving either placebo, or 40 mg of MKS492 (Table 2).

With oral placebo, allergen challenge caused a rapid fall in FEV₁ (from post-saline baseline) to a mean maximum decrease in FEV₁ of 75.9% (12.7). This was followed in five subjects by a gradual recovery to within 5% of baseline by 2 h and no late fall ($\geq 15\%$) in FEV₁ occurred. These subjects were classified as single early responders. The remaining 13 subjects subsequently developed a late phase or protracted bronchoconstrictor response 2–8 h after challenge with a fall in FEV₁ of $\geq 15\%$ from post-saline baseline (Table 1). The mean maximum decrease in FEV₁ during the late phase in these dual-responders was 66.5% (15) of post-saline baseline FEV₁ measurements.

Early asthmatic responses

Using all 18 subjects, no differences were found between placebo and 20 mg MKS492 for any of the early response outcome measures studied. Table 3 shows means of summary FEV₁ outcome measures compared between the three treatment groups for the 12 subjects receiving all treatments. At 30 min, the mean % of post saline FEV₁ was 81.6 (14.0) for placebo, 81.3 (11.9) for 20 mg MKS492 and 93.1 (13.8) for 40 mg MKS492. The groups were significantly different overall ($P=0.017$): the 40 mg dose resulting in a % of post saline FEV 11.5% (95% CI for difference: 2.6, 20.4) higher than placebo and 10.0% (95% CI for difference: 1.1, 18.9) higher than 20 mg. The mean minimum % of post saline

FEV₁ up to 30 min was also increased by the 40 mg dose (80.0 [14.1], 79.3 [12.0] and 88.7 [12.0] for placebo, 20 mg and 40 mg respectively), but the differences between groups did not reach statistical significance ($P=0.06$). At 120 min, the mean % of post saline FEV₁ was 86.5 (12.0) for 40 mg MKS492 compared with 78.5 (12.0) for 20 mg and 78.0 (13.9) for placebo ($P=0.09$). Analysis of the area under the FEV₁ response-time curve (AUC) also suggested a reduction in the early phase (0–2 h) of bronchoconstriction with 40 mg MKS492, but again the difference between treatments was not significant ($P=0.3$).

Late asthmatic response

Analysis of the late phase outcomes was restricted to the 13 subjects who demonstrated a late response to allergen challenge. There was no difference between placebo and 20 mg MKS492 for the late phase response summary measures. Results for the late phase responses for the subjects receiving all three treatments are also shown in Table 3. The mean % of post saline FEV₁ at 240 min was 83.0 (12.5) for placebo, 79.6 (10.0) for 20 mg and 90.0 (6.3) for 40 mg MKS492. The overall difference was significant ($P=0.018$), with the 40 mg dose causing an increase of 7.0% (95% CI for difference: 0.1, 13.9) above placebo and 10.4% (95% CI for difference: 3.5, 17.3) above 20 mg. The mean minimum % of post saline FEV₁ up to 450 min was 66.5 (15.0) for placebo, 66.2 (16.0) for 20 mg and 75.9 (8.7) for 40 mg ($P=0.118$). The area under the FEV₁ time-response curve also showed evidence of an effect of 40 mg MKS492, but the differences were not significant ($P=0.158$).

Analysis of absolute changes in FEV₁

Because baseline FEV₁ had been affected by treatment (Table 2), lung function was also analysed as the absolute post-allergen FEV₁ values (as opposed to % change from post-saline FEV₁). Similar trends were observed using either method (Figures 1 and 2), but differences between placebo and MKS 492–20 mg were now more marked as were differences between placebo and 40 mg (Table 4). This was true for both early and late asthmatic responses. However, the shape of the FEV₁ response-time curves were similar to that observed for post-saline FEV₁ changes, suggesting little inhibition of late phase responses beyond the higher baseline.

Cardiovascular and safety parameters

No significant adverse effects occurred in association with both doses of MKS492. Headache was reported in three cases (one severe) and dizziness in one subject. Measurements of pulse rate and blood pressure were unchanged, although extrasystolic beats were noted in one asymptomatic subject after 40 mg MKS492. Electro-cardiographic evaluation was normal in all subjects except for ventricular extrasystolic complexes seen in the asymptomatic patient who had an irregular pulse rhythm. Likewise, there were no significant changes in haematological parameters and hepatic functions.

Table 2 Change in baseline FEV₁ after placebo MKS492 20 mg 40 mg.

Treatment	Baseline mean(s.d.)	+ 90 min mean(s.d.)	% Change mean(s.d.)
Placebo	3.3 (0.8)	3.0 (0.9)	−7.5 (6.6)
MKS492 20 mg	3.1 (0.8)	3.1 (0.9)	−0.3 (6.4)
MKS492 40 mg	3.1 (1.0)	3.4 (1.0)	+11.5 (12.0)*

* $P=0.001$ vs placebo and 20 mg.

Table 3 Percentage of post saline FEV₁ and auc during the early and late phases following allergen challenge.

Measurement	Placebo	20 mg mean(s.d.)	40 mg mean(s.d.)	Overall P value (ANOVA)	P value placebo vs 40 mg	P value 20 mg vs 40 mg
FEV ₁ at 30 min	81.6(14.0)	83.1(11.9)	93.1(13.8)	0.017	0.014	0.011
Max decrease	80.0(14.1)	79.3(12.0)	88.7(12.0)	0.069		
FEV ₁ to 30 min	42.6(45.5)	45.6(37.7)	26.9(15.0)	0.301		
AUC to 120 min						
Max decrease	78.0(13.9)	78.5(12.0)	86.5(12.0)	0.096		
FEV ₁ to 120 min	83.0(12.5)	79.6(10.0)	90.0 (6.3)	0.018	0.048	0.006
FEV ₁ at 240 min +						
Max decrease	66.5(15.0)	66.2(16.0)	75.9 (8.7)	0.118		
FEV ₁ to 450 min	159.0(134.9)	197.4(98.1)	128.1(81.2)	0.158		
AUC to 450 min						

*FEV₁ expressed as % of post-saline FEV₁.

+ Late phase responders only.

AUC, Area under the FEV₁ response-time curve.

Figure 1 FEV₁ response-time course curves after placebo (Δ) and MKS492 40 mg ($\square$). Significant inhibition of the early asthmatic response is present with attenuation of late responses. FEV₁ values are expressed as % of post-saline baseline FEV₁.

Figure 2 FEV₁ response-time course curves using FEV₁ absolute values. Baseline increases in FEV₁ in the MKS492 40 mg group were observed which were maintained during both the early and late phase responses. The shape of the time course curves is similar to that observed for % post-saline changes, suggesting little benefit additional to bronchodilatation at baseline. Δ placebo, $\square$ MKS492 40 mg.

Table 4 Measurements of FEV₁ in 12 patients following allergen challenge.

Measurement (l)	Placebo mean(s.d.)	20 mg mean(s.d.)	40 mg mean(s.d.)	Overall P value (ANOVA)	P value placebo vs 40 mg	P value 20 mg vs 40 mg
FEV ₁ at 30 min	2.48(0.83)	2.65(0.92)	3.02(1.13)	0.003	0.001	0.015
Min FEV ₁ to 30 min	2.38(0.84)	2.54(0.85)	2.86(1.12)	0.022	0.007	0.061
Min FEV ₁ to 120 min	2.34(0.85)	2.52(0.84)	2.81(1.12)	0.096	—	—
FEV ₁ at 240 min +	2.74(1.03)	2.86(1.01)	3.21(1.10)	0.001	<0.001	0.004
Min FEV ₁ to 450 min +	2.17(0.82)	2.41(1.8)	2.73(1.05)	0.026	0.008	0.097

+ Late phase responders only.

FEV₁ Forced expiratory volume in 1 s.

All data shown reflects absolute FEV₁ measurements.

Discussion

We have examined the pharmacological effects of a novel PDE 3 inhibitor in both the early and late bronchoconstrictor phases of asthma. The higher dose (40 mg) of this compound, given as a single dose, afforded significant protection against the immediate bronchoconstriction induced by allergen and attenuated the development of a late response.

Although PDE inhibitors have been evaluated clinically in a number of disorders, few studies have examined their effects on airway function in asthma. Use of a bronchodilator and anti-inflammatory compound as a single drug for asthma is an attractive concept; an idea supported by the history of therapeutic efficacy of theophylline, a non-selective PDE inhibitor. Because theophylline antagonises adenosine receptors causing gastrointestinal, central nervous system and cardiovascular side effects, the development of drugs that couple the efficacy of theophylline with an improved safety profile and increased therapeutic index, would be an important advance.

Clinical studies of selective PDE inhibitors have been inconclusive thus far. An early study of the PDE 4 inhibitor, tiabendazole, showed no significant improvements in airway function after oral administration [6]. Similarly, inhaled zardaverine, a PDE 3 and 4 inhibitor, exhibited only modest and short-lasting bronchodilating activity [7]. The PDE 4 inhibitor, zaprimast, afforded some protection against exercise-induced asthma in adults [8], but not in children [9].

MKS492 is a selective PDE 3 inhibitor that has shown potential both as a bronchodilator and as an anti-inflammatory agent. A single dose of either 20 mg or 40 mg was administered and the effects on both early and late asthmatic responses were assessed. The lower dose of MKS492 (20 mg) had no protective effects and values were closely matched with that of placebo. In contrast, the higher dose of 40 mg had a significant influence on the early response as measured by the decrease in FEV₁ at 30 min post-allergen; there was also a trend for a reduction in maximum mean decrease in FEV₁ and area under the FEV₁ time-response curves. This observation may tally with the reported potent smooth muscle relaxing activities of PDE 3 inhibitors [10, 11]. A similar pattern was observed in late phase responses with similar placebo and 20 mg values contrasted to improved responses associated with the 40 mg dose of MKS492. Although analysis of changes in absolute FEV₁ demonstrated greater degrees of protection against allergen-induced bronchoconstriction, the response curves were parallel suggesting little additional benefit beyond the baseline bronchodilatation given by MKS492.

Selective xanthine analogues do not antagonise adenosine receptors, but may have potent smooth muscle relaxing properties and cause hypotension and cardiovascular instability. This was carefully evaluated in the study and a single serious adverse reaction was noted. In this patient an irregular pulse (without hypotension and which disappeared after 3–4 h) was observed; on electrocardiographic evaluation the arrhythmia appeared to reflect ventricular extrasystolic

beats. Headache was noted in a few subjects but hepatic and haematological measurements were unchanged.

In summary, we have evaluated the effects of a novel PDE 3 inhibitor on the early and late asthmatic responses. Early responses were significantly diminished, whilst late responses overall were attenuated, but not entirely prevented. It suggests that this group of selective compounds may have a role to play in the treatment of asthma and should encourage longer term clinical studies using higher doses of this and other selective PDE inhibitors.

This work was supported by a post-doctoral SA MRC Fellowship to PGB, Internal Medicine, University of Stellenbosch and Sandoz Pharma Ltd, Basle. We should like to thank all our asthmatic volunteers who participated in the study and Mark Bulpitt who helped with data analysis. Expert secretarial assistance was provided by Maggie Dowling and Susan Stipp.

References

- 1 Rabe TJ, Magnussen H, Dent G. Theophylline and selective PDE inhibitors as bronchodilators and smooth muscle relaxants. *Eur Respir J* 1995; **8**: 637–642.
- 2 Raeburn D, Souness JE, Tomkinson A, Karlsson J-A. Isozyme selective cyclic nucleotide phosphodiesterase inhibitors: Biochemistry, pharmacology and therapeutic potential in asthma. *Prog Drug Res* 1993; **40**: 9–32.
- 3 Turner CR, Andresen CJ, Smith WB, Watson JW. Effects of rolipram on responses to acute and chronic antigen exposure in monkeys. *Am J Respir Crit Care Med* 1994; **149**: 1153–1159.
- 4 Morley J, Chapman ID, Mazzoni L. SDZ MKS492. *Agents Actions* 1991; **34**: 403–410.
- 5 Giembycz MA, Dent G. Prospects for selective cyclic nucleotide phosphodiesterase inhibitors in the treatment of bronchial asthma. *Clin Exp Allergy* 1992; **22**: 337–344.
- 6 Israel E, Mathur PN, Tashkin D, Drazen JM. LY 186655 prevents bronchospasm in asthma of moderate severity. *Chest* 1988; **91**: 715–718.
- 7 Brunnée T, Engelstätter R, Steinjans VW, Kunkel G. Bronchodilatory effect of inhaled zardaverine, a phosphodiesterase III and IV inhibitor, in patients with asthma. *Eur Respir J* 1992; **5**: 982–985.
- 8 Rudd RM, Gellert AR, Studdy PR, Geddes DM. Inhibition of exercise-induced asthma by an orally absorbed mast cell stabiliser. *Br J Dis Chest* 1983; **77**: 78–86.
- 9 Reiser J, Yeang Y, Warner JO. The effect of zaprimast, an orally absorbed mast cell stabiliser on exercise-induced asthma in children. *J Dis Chest* 1986; **80**: 157–163.
- 10 Giembycz MA. Could isoenzyme-selective phosphodiesterase inhibitors render bronchodilator therapy redundant in the treatment of bronchial asthma? *Biochem Pharmacol* 1992; **43**: 2041–2051.
- 11 Fujimura M, Kamio Y, Saito M, Hashimoto T, Matsuda T. Bronchodilator and bronchoprotective effects of cilostazol in humans *in vivo*. *Am J Respir Crit Care Med* 1995; **151**: 222–225.

(Received 15 April 1997,
accepted 29 September 1997)