

Rapid effect of inhaled fluticasone propionate on airway responsiveness to adenosine 5'-monophosphate in mild asthma

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Inhaled adenosine 5'-monophosphate (AMP) has an "indirect" bronchoconstrictive effect through mast cell degranulation and mediator release, whereas inhaled histamine has a "direct" effect on smooth muscle. Prolonged treatment with inhaled glucocorticosteroids attenuates airway responsiveness (AR) to AMP and histamine. We investigated the early effects of inhaled fluticasone propionate (FP) therapy on AR in 3 consecutive double-blind, randomized, placebo-controlled crossover studies in steroid-naïve subjects with mild asthma. In one study, each of 12 subjects received FP 1000 µg or matched placebo for 7 inhalations at 12 hourly intervals; AR to AMP and FEV₁ were measured 2 hours after the 3rd and 7th inhalations. In a second study, each of 12 subjects received FP 100, 250, or 1000 µg or matched placebo for 3 inhalations at 12 hourly intervals; AR to AMP and FEV₁ were measured 2 hours after the 1st and 3rd inhalations. In a third study, each of 8 subjects received a single inhalation of FP 1000 µg or matched placebo; AR to histamine was measured 2 hours later. In the first study, FP 1000 µg significantly attenuated AR to AMP by 2.7 and 2.5 doubling doses after 3 and 7 inhalations, respectively ($P \leq .0001$). In the second study, FP 100, 250, and 1000 µg significantly attenuated AR to AMP by 1.9, 2.2, and 2.7 doubling doses, respectively, after 1 inhalation and by 2.4, 2.2, and 3.2 doubling doses, respectively, after 3 inhalations ($P \leq .0001$); a small but significant increase in FEV₁ (>0.15 L) was observed after 3 inhalations but not after 1 inhalation of FP irrespective of dose ($P \leq .05$). In the third study, a single inhalation of FP 1000 µg had no effect on AR to histamine. We have demonstrated a reduction in AR to AMP but not AR to histamine within 2 hours of a single inhalation of FP. This reflects a rapid, topical anti-inflammatory action of inhaled FP by a mechanism of action that remains unknown. (J Allergy Clin Immunol 2002;110:603-6.)

Key words: Adenosine 5'-monophosphate, AMP, fluticasone propionate, induced bronchoconstriction, atopic asthma, asthma, airway responsiveness, airway mast cells

The major bronchoconstrictive effect of inhaled adenosine 5'-monophosphate (AMP) follows in vivo conversion to adenosine with consequent A2b receptor-mediated mast cell activation, degranulation, and mediator release. Measurement of airway responsiveness (AR) to this "indirect-acting" smooth muscle spasmogen might be a more useful and sensitive marker of allergic airway inflammation in asthma than measurement of AR to the "direct-acting" spasmogen histamine or to methacholine.¹

Inhaled glucocorticosteroids (GCSs) are the most effective anti-inflammatory therapy currently available for asthma, though their mechanism of action is not fully understood. We have previously demonstrated that regular inhaled budesonide reduces AR to AMP to a significantly greater extent than it reduces AR to methacholine in subjects with mild asthma.² This might represent a reduction in mast cell numbers and/or a reduction in function. We have also demonstrated that regular ciclesonide, a novel GCS, attenuates AR to AMP in a dose-dependent manner.³ The speed of onset of this reduction in AR to AMP following inhaled GCS therapy remains unknown.

To assess the early effects of inhaled fluticasone propionate (FP), we compared the effects of 3 and 7 inhalations of FP 1000 µg administered at 12 hourly intervals on AR to AMP and FEV₁. The results of this study suggested a more rapid effect of FP on AR to AMP. A second study therefore assessed AR to AMP and FEV₁ after both a single inhalation and 3 inhalations of FP 100, 250, and 1000 µg, also administered at 12 hourly intervals. We also assessed AR to histamine after a single inhalation of FP 1000 µg.

SUBJECTS, MATERIALS, AND METHODS

Thirty-eight nonsmoking subjects aged 19 to 36 years were enrolled into the 3 studies. Each had mild asthma (FEV₁ $> 70\%$ of predicted), a history of atopy, and demonstrated sensitivity to AMP at screening, with a PC₂₀ of <50 mg/mL. Each subject also had infrequent symptoms controlled with occasional inhaled short-acting β_2 -agonists alone, and each had not used GCS for at least 3

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Abbreviations used

AMP: Adenosine 5'-monophosphate
AR: Airway responsiveness
DD: Doubling dose
FP: Fluticasone propionate
GCS: Glucocorticosteroid

months before entry. Written informed consent was obtained from all subjects, and the studies were approved by the Ethics Committee of King's College Hospital. All studies were randomized, double-blind, placebo-controlled, and crossover in design. Subjects refrained from using rescue medication and caffeinated beverages for at least 8 hours before each visit.

Study 1: Effect of 3 and 7 inhalations of FP 1000 µg on AR to AMP

Each of 16 subjects received either FP 1000 µg or matched placebo at 12 hourly intervals via an Accuhaler (GlaxoSmithKline, UK) for 7 inhalations. AMP challenge was performed as previously described^{2,3} at baseline, 2 hours after the 3rd inhalation, 2 hours after the 7th inhalation, and 7 days and 14 days later during the washout period.

Study 2: Effect of 1 and 3 inhalations of FP 100, 250, and 1000 µg on AR to AMP

Each of 14 subjects received either FP 100, 250, or 1000 µg or matched placebo at 12 hourly intervals for 3 inhalations. AMP challenge was performed at baseline, 2 hours after the 1st inhalation, 2 hours after the 3rd inhalation, and after a 7-day washout period.

Study 3: Effect of a single inhalation of FP 1000 µg on AR to histamine

Each of 8 subjects received either a single inhalation of FP 1000 µg or matched placebo. A histamine challenge was performed 2 hours later.

RESULTS

Baseline characteristics were comparable in each study.

Effect of FP on AR to AMP

Study 1: Effect of 3 and 7 inhalations of FP 1000 µg. A total of 12 subjects completed the study. FP significantly reduced AR to AMP by 2.7 (95% CI, 1.9 to 3.4) doubling doses (DDs) after 3 inhalations ($P \leq .0001$) and by 2.5 (95% CI, 1.8 to 3.2) DDs after 7 inhalations ($P \leq .0001$) in comparison with placebo. The effect disappeared within 7 days (Fig 1, A).

Study 2: Effect of 1 and 3 inhalations of FP 100, 250, and 1000 µg. A total of 12 subjects completed the study. FP significantly reduced AR to AMP after each treatment by 1.9 (95% CI, 1.0 to 2.8), 2.2 (95% CI, 1.3 to 3.2), and 2.7 (95% CI, 1.8 to 3.7) DDs after 1 inhalation ($P \leq .0001$) and by 2.4 (95% CI, 1.5 to 3.3), 2.2 (95% CI, 1.3 to 3.1), and 3.2 (95% CI, 2.3 to 4.1) DDs after 3 inhalations ($P \leq .0001$) of FP 100, 250, and 1000 µg, respectively (Fig 1, B). Although there was a trend toward a greater effect for FP 1000 µg than for lower doses, this

did not achieve significance. There was no statistical difference between the effect of 1 inhalation and the effect of 3 inhalations of FP irrespective of the dose.

Effect of FP on FEV₁

There was no carryover effect between treatment periods and no significant difference in baseline FEV₁ values before each treatment period in each study.

Study 1. There were small but significant increases in FEV₁ of 0.19 L after 3 inhalations of FP 1000 µg ($P = .02$), 0.17 L after 7 inhalations ($P = .04$), and 0.16 L 7 days later ($P = .05$; Fig 2, A).

Study 2. There were small but significant increases in FEV₁ of 0.15 L ($P = .01$), 0.18 L ($P = .004$), and 0.17 L ($P = .0006$) after 3 inhalations of FP 100, 250, and 1000 µg, respectively, in comparison with placebo. No significant difference was seen after a single inhalation of any dose (Fig 2, B).

Effect of FP on AR to histamine

A total of 8 subjects completed study 3, in which we investigated the effect of a single inhalation of FP 1000 µg. FP had no effect on AR to histamine ($P = .8$).

DISCUSSION

Our studies are the first to demonstrate rapid inhibition of AMP responsiveness following inhalation of FP 100 to 1000 µg in mild stable asthma. Significant attenuation occurs 2 hours after a single inhalation of FP 100 µg. There was a statistically significant, albeit small, improvement in FEV₁ after 3 inhalations but not after 1 inhalation of FP 100 to 1000 µg. There was no additional benefit of 3 or 7 inhalations over 1 inhalation of FP 1000 µg on AMP PC₂₀, suggesting that the effect is not cumulative.

AMP induces mast cell degranulation and release of mediators, including histamine, prostanoids, and eicosanoids. This results in airway narrowing due to smooth muscle constriction and mucosal edema as a result of increased mucosal blood flow and microvascular permeability. AMP might also act on A2b receptors in vascular beds and neurosecretory cells to induce mucosal edema directly.¹

Previously, we had assumed that prolonged GCS decreases AR to AMP responsiveness by reducing mast cell numbers and/or function.^{2,3} We have now shown an equivalent change in AR after a single inhalation of FP. There is no evidence to suggest that a single inhalation of GCS will reduce mast cell number or function.⁴ Inhibition of mast cell mediator responses might offer a better explanation of our results. However, we have failed to show any effect of a single inhalation of FP 1000 µg on AR to histamine.

There have been no direct measurements of acute inhaled GCS effects on airway microvascular permeability in subjects with asthma. In animal studies, airway microvascular permeability is inhibited within 2 to 4 hours of single-dose GCS administration.⁵ A single

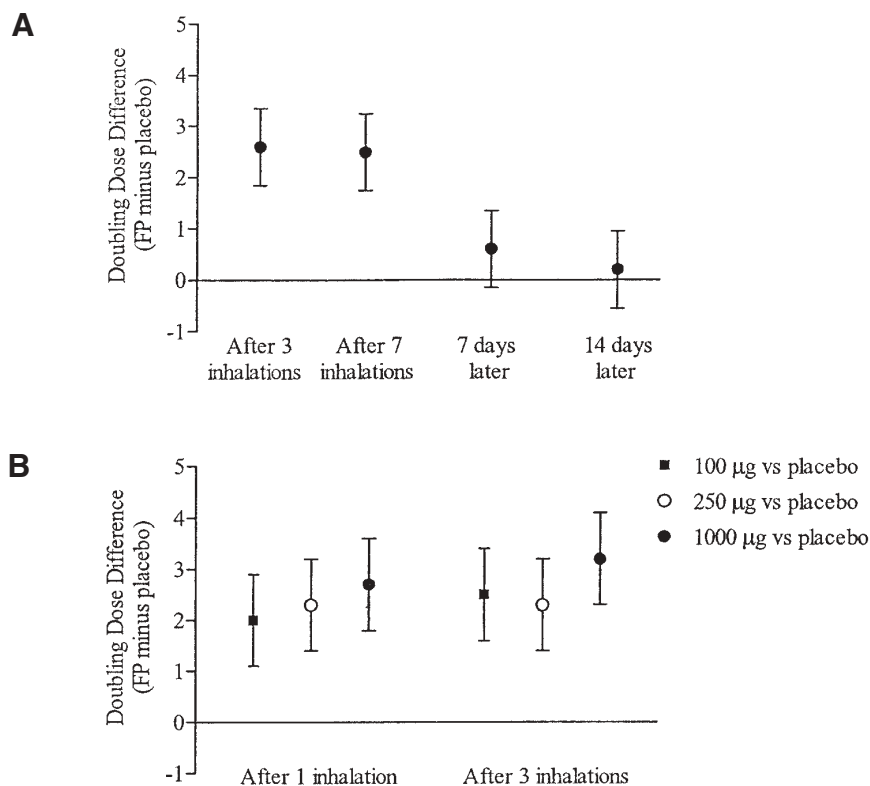


FIG 1. Comparisons of AR to AMP (PC_{20}) after FP and placebo treatment. Differences (FP minus placebo) and 95% CI after 3 and 7 inhalations of FP 1000 µg (**A**) and after 1 and 3 inhalations of FP 100, 250, and 1000 µg (**B**) are expressed in terms of DD difference ($n = 12$). FP significantly reduced AR to AMP after each treatment. $P \leq .0001$ by ANOVA.

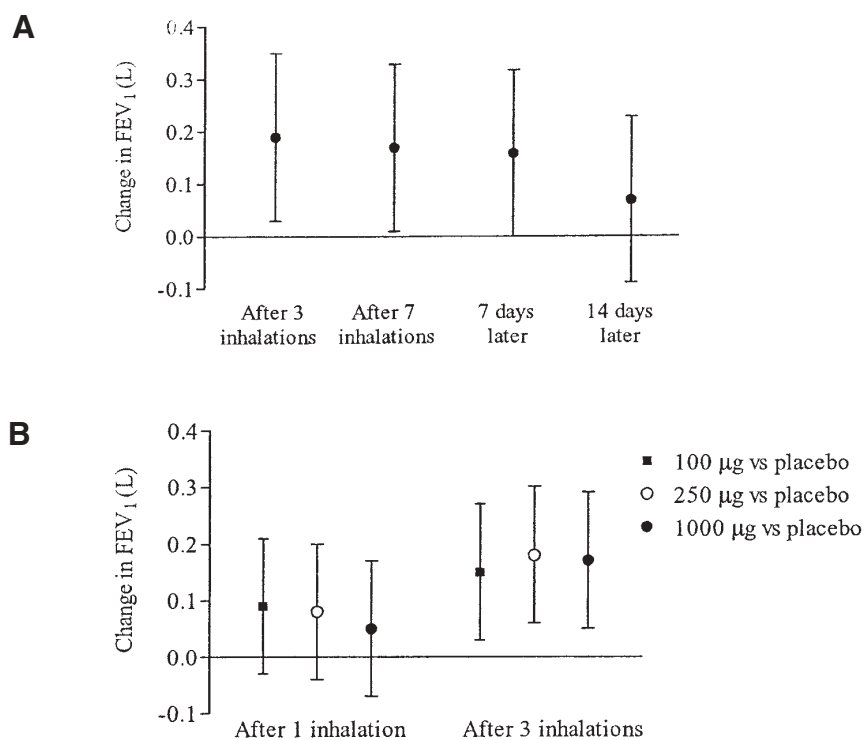


FIG 2. Changes in FEV_1 . Differences (FP minus placebo) and 95% CI after 3 and 7 inhalations of FP 1000 µg (**A**) and after 1 and 3 inhalations of FP 100, 250, and 1000 µg (**B**) are presented. A small but significant increase in FEV_1 is demonstrated after 3 inhalations of each dose ($P \leq .05$ by ANOVA) but not after 1 inhalation of each dose.

inhalation of FP reduces airway mucosal blood flow, possibly as a result of its vasoconstrictive effects.⁶ Such phenomena might partly explain our results—perhaps through inhibition or functional antagonism of A2b receptor-mediated effects on airway microvascular permeability or airway mucosal blood flow. Indeed, the late asthmatic response, which might in part be due to airway wall edema, is inhibited by a single inhalation of GCS, administered after allergen challenge.⁷

A rapid effect of FP on mechanisms other than mast cell-mediated events should be considered. Classically, the major action of GCS involves binding to an intracellular receptor, leading to modulation of transcriptional and translational events. These genomic effects take several hours, but they might explain our results. A single dose of intranasal budesonide inhibits allergen-induced nasal mucosal output of GM-CSF and IL-5 within 3 hours.⁸ A rapid, nongenomic effect of GCS that can occur within minutes is a plausible alternative, possibly by modifying adenosine receptors at a cell membrane level and/or by altering the activation of second messenger/intracellular signaling pathways.⁹

Although we chose well-controlled, steroid-naïve patients with mild intermittent asthma, even FP 100 µg reduced AR to AMP well beyond the variability of the challenge. The repeatability of the baseline challenges was within 0.45 DDs. With near-optimal FEV₁ baseline values, we were also surprised to see statistically significant improvements (albeit only 0.15 to 0.19 L) after 3 inhalations of FP. The mechanisms involved in attenuating AR to AMP and increasing baseline FEV₁ in our studies might be of greater clinical significance in acute allergic asthma. Available evidence suggests that systemic GCS require 6 to 24 hours to have any beneficial effect on FEV₁ in acute asthma. However, high-dose inhaled flunisolide has an effect on FEV₁ within 2 hours.¹⁰

Our study demonstrates that inhaled FP 100 to 1000 µg attenuates AR to AMP within 2 hours by a mechanism of action that remains unknown; this confirms an acute anti-inflammatory effect of inhaled GCS. The use of inhaled GCS in acute asthma exacerbations might provide additional beneficial effects of topically applied GCSs when added to systemic GCSs and bronchodilators.

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