

Prolonged protection of the new inhaled corticosteroid fluticasone furoate against AMP hyperresponsiveness in patients with asthma

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Abstract

Introduction: Single-dose inhaled corticosteroids (ICS) induce direct anti-inflammatory effects in asthma thereby improving airway hyperresponsiveness (AHR). A novel enhanced-affinity ICS, fluticasone furoate (FF), demonstrated a prolonged duration of action *in vitro*. The aim of this study was to evaluate the efficacy and duration of action of a single dose of FF by studying protection against AHR to adenosine 5'-monophosphate (AMP) and effects on exhaled nitric oxide (eNO).

Methods: A randomized, double-blind, placebo-controlled, 6-way crossover study (FFA10026) was performed in 24 patients with allergic asthma (mean age 32.8 years, FEV₁ ≥ 70% predicted and PC₂₀ AMP ≤ 50 mg/ml). Each subject received a single dose of FF 1000 µg, fluticasone propionate (FP) 1000 µg, or placebo at 2 (FF only), 14 or 26 h prior to AMP challenge and eNO measurement.

Results: FF significantly improved PC₂₀ AMP compared to placebo, the difference in doubling concentrations being 2.18 (95% confidence interval: 1.13–3.23), 1.54 (0.48–2.59), and 1.30 (0.26–2.34) at 2, 14 and 26 h. FP improved PC₂₀ AMP significantly at 14 h compared to placebo, but not at the 26-hour time point, the difference in doubling concentrations being 1.72 (0.70–2.75) and 0.33 (–0.69–1.34). There was no significant effect on eNO after either FF or FP at all time points. FF was well tolerated and there were no serious adverse events.

Conclusion: The new inhaled corticosteroid FF, but not FP, demonstrates prolonged protection up to 26 h against AHR to AMP in asthma patients.

Asthma is a disease characterized by airway inflammation and recurrent episodes of symptoms of wheezing and chest tightness that are associated with variable airway obstruction and airway hyperresponsiveness (AHR) (1). Inhaled corticosteroids (ICS) are the cornerstone of asthma treatment, because they suppress inflammation thereby decreasing asthma symptoms, the number of exacerbations and improving lung function and AHR (2, 3). The severity of AHR can be measured with histamine or methacholine. Both agents are direct stimuli, because they exert their effects directly on airway smooth muscle cells. Another possible stimulus to measure AHR is adenosine 5'-monophosphate (AMP). AMP is an indirect stimulus, because it acts indirectly via the

release of inflammatory mediators. It has been shown in several studies that the provocative concentration of AMP causing the forced expiratory volume (FEV₁) to drop by 20% (PC₂₀) is a better tool to measure the anti-inflammatory effects of ICS than the PC₂₀ methacholine or PC₂₀ histamine (4, 5). The protective effect of ICS on AMP-induced bronchoconstriction is rapid and starts already 2 h after administration of a single-dose fluticasone propionate (FP) (6). In addition, it has been demonstrated that the PC₂₀ AMP is a useful tool to investigate the duration of action of a single inhaled dose of FP (7).

Recently, a new ICS has been developed for the treatment of asthma: fluticasone furoate (FF). Fluticasone furoate was

identified after detailed investigation of the effects of changing the 17- α ester group of FP. Although FF and FP are structurally related, they are distinct chemical and pharmacological entities. The 17- α ester group of FF and FP is metabolically stable and is not cleaved from the rest of the molecule. Fluticasone furoate and FP share no common metabolites, and neither compound is metabolized to fluticasone. Fluticasone furoate is therefore a distinct drug molecule and not a salt or a prodrug of fluticasone. Fluticasone furoate has a greater affinity for the glucocorticoid receptor than FP. In addition, 'in vitro' studies have shown a greater protection against elastase-induced loss of bronchial epithelial integrity and a prolonged retention in respiratory epithelial cells of FF when compared to FP, budesonide or mometasone furoate (8). This is important, because it suggests that FF may provide a more effective treatment in asthma.

This study aims to investigate the efficacy and duration of action of FF in patients with asthma. To this end, we performed a randomized double-blind, placebo-controlled 6-way crossover study to compare effects of a single-dose FF, FP, or placebo when given at different time points before assessing PC₂₀ AMP measuring of exhaled nitric oxide (NO).

Material and methods

Patients

Twenty-four asthmatics were included if they fulfilled the following inclusion criteria: a positive history of asthma, FEV₁ > 70% predicted, PC₂₀ AMP < 50 mg/ml, the presence of atopy (i.e. at least one positive skin prick tests to the following aeroallergens: house dust mite, cat, grass, trees or aspergillus), and age between 18 and 55 years. All subjects were nonsmokers at entry to the study; they were not allowed to have smoked during the last 12 months and not more than a maximum of 10 pack years preceding the study. Patients were not allowed to use ICS or any of the following medication: oral corticosteroids within 8 weeks before the screening visit, inhaled, intranasal or topical corticosteroids within 4 weeks before the screening visit, antihistaminics within 2 weeks before the screening visit, and NSAID's, nasal decongestants, cromolyn sodium, nedocromil sodium less than 1 week before screening visit. Furthermore, long-acting β_2 -agonists were not allowed for 96 h prior to the start of the study. Patients did not have respiratory infections within 6 weeks before study entry. All patients gave their written informed consent, and the study was approved by the medical ethic committees of each centre.

Design of the study

The study was a three-centre, randomized, double-blind, placebo-controlled, 6-way crossover study (FFA10026). After the screening visit, all patients underwent a second AMP challenge at least 48 h later to demonstrate stability of their PC₂₀ AMP as described in previous studies (7, 9). If the second PC₂₀ AMP was within 1.25 doubling concentrations of the first AMP challenge, patients were included in the

study. Then, they were randomized to receive each of the six following treatment regimens:

Regimen A: Placebo.

Regimen B: FF 1000 μ g 2 h prior to measurement of eNO and PC₂₀ AMP.

Regimen C: FF 1000 μ g 14 h prior to measurement of eNO and PC₂₀ AMP.

Regimen D: FF 1000 μ g 26 h prior to measurement of eNO and PC₂₀ AMP.

Regimen E: FP 1000 μ g 14 h prior to measurement of eNO and PC₂₀ AMP.

Regimen F: FP 1000 μ g 26 h prior to measurement of eNO and PC₂₀ AMP.

Because of the large crossover design including 6 treatment periods, it was decided not to measure the 2-hour time point for FP since this had been studied previously (7). Each treatment period was separated by at least 5 days and a maximum of 10 days. Throughout the study, patients were allowed to use inhaled salbutamol 100 μ g per puff as rescue medication. However, they were not allowed to have used salbutamol for at least 8 hours before each visit to the hospital.

Measurement of PC₂₀ AMP

AMP (Sigma-Aldrich, St. Louis, MO, USA) was inhaled at 5-min intervals in doubling concentrations starting from 0.04 mg/ml to a maximum of 320 mg/ml according to a standardized protocol as described previously (10). After inhalation of AMP during two minutes of tidal breathing from a calibrated (0.13 ml/min) nebulizer (model 646, Devilbiss Inc., Somerset, PA, USA), FEV₁ was measured in duplicate at 30 and 90 s. AMP was given until the FEV₁ fell by at least 20% from baseline FEV₁ or when the maximum concentration of AMP was inhaled.

FEV₁ and exhaled nitric oxide measurements

All lung function measurements were performed with the same spirometer (mobile bi-directional digital spirometer Sensorloop, SensorMedics Corporation, Yorba Linda, Ca, USA or a Jaeger Masterscope, Hoechberg, Germany). A calibrated chemiluminescence nitric oxide analyser was used to measure exhaled nitric oxide (eNO) during the study according to ATS standards at the same time of day for all treatment periods. As each centre used its own eNO analyser, three different types were used: LR2500 Logan (Research Ltd, Rochester, Kent, UK), or the ECO physics, type CLD 700 AL or CLD77AM (ECO physics, Dürnten, Switzerland). The average of three acceptable measurements was recorded. All eNO recordings were performed prior to assessment of FEV₁ values.

Data analysis

The study was statistically powered to detect a doubling concentration difference of 1.5 between any of the active doses and placebo. This difference is approximately equal to the previously observed effect by FP 1000 μ g 14 h prior to the

AMP challenge (7). Assuming a relative conservative standard deviation of 1.25, we would need 18 subjects to detect a doubling concentration difference of 1.5 between any of the active treatments and placebo with 90% power using a 2-sided 5% significance level. As we expected a dropout of approximately 25% (as a result of the six-way crossover design), it seemed reasonable to randomize 24 subjects. The PC₂₀ AMP and eNO measurements were analysed using a mixed effects model with the subject fitted as random effect. The following fixed effects were included in the models: period and treatment group. The data were log (base 2) transformed prior to analyses. For the PC₂₀ AMP, differences and 95% confidence intervals between treatment groups are presented in terms of doubling doses. For the eNO data, the comparisons are presented in terms of a ratio and 95% confidence intervals (FF/placebo or FP/placebo). Differences were considered to be statistically significant when the 2-sided *P*-value was below 0.05.

Results

Included subjects and adverse events

Twenty-four asthma patients were randomized and 18 completed the study. Baseline characteristics are presented in Table 1. Six patients withdrew from the study: two withdrew their consent, one started to use antihistamines for allergic complaints and one was withdrawn because of a protocol violation (FEV₁ < 70% predicted at the start of the study).

Table 1 Baseline patient characteristics*

Mean age	32.8 ± 11.8
Male/female	23/1
Current smokers, <i>n</i>	0
FEV ₁ , L	3.94 ± 0.69
FEV ₁ % predicted	93.3 ± 13.3
FEV ₁ /FVC (%)	75 ± 9
PC ₂₀ AMP (mg/ml)†	6.4 (0.5–47)
Exhaled nitric oxide (ppm)‡	41.9–45.3

AMP, adenosine 5'-monophosphate.

*Data are presented as mean ± SD unless specified otherwise.

†Expressed as geometric mean and range.

‡As no eNO measurement was performed at screening, the range of predose geometric means at each of the six treatment periods is presented.

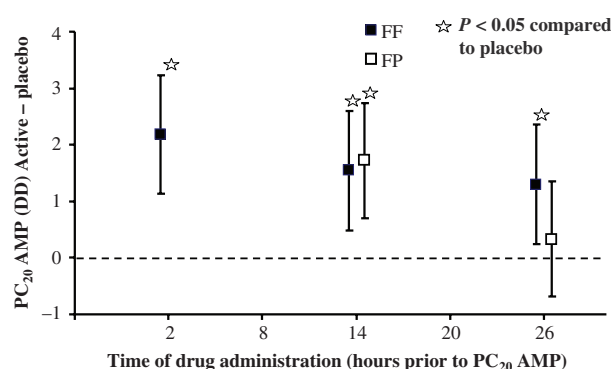


Figure 1 Change in PC₂₀ adenosine 5'-monophosphate AMP expressed as doubling doses (mean with 95% confidence interval) 2, 14 and 26 h after a single dose of FF or FP.

Furthermore, two subjects were withdrawn after an adverse event (one patient experienced dizziness after an AMP challenge and one patient suffered from an asthma exacerbation for which oral prednisolone was prescribed).

Protective effect against the PC₂₀ AMP and eNO

Fluticasone furoate significantly improved the PC₂₀ AMP at all time points compared to placebo, the mean (95% confidence interval) difference in doubling concentrations being 2.18 (1.13–3.23), 1.54 (0.48–2.59), and 1.30 (0.26–2.34) at 2, 14, and 26 hours, respectively (Fig. 1 and Table 2). Fluticasone propionate significantly improved the PC₂₀ AMP at 14 h but not at 26 h compared to placebo, the difference in doubling concentrations being 1.72 (0.70–2.75) and 0.33 (-0.69–1.34) at 14 and 26 h respectively. No significant changes in the concentration of eNO were observed after FF or FP treatment at any time point (Table 2). Finally, no relationships were found between the level of FEV₁ and improvement in PC₂₀ AMP 2, 14 or 26 h after treatment with FP, FF or placebo.

Adverse events of FF and FP

Of the 18 subjects who completed the study, a total of 44 adverse events were reported and there were no serious adverse events. The most frequently occurring adverse event was bronchospasm (33%), followed by dyspnea, dizziness,

Table 2 Changes in PC₂₀ adenosine 5'-monophosphate (AMP) and nitric oxide after treatment with FF or FP and Placebo*

	Fluticasone Furoate			Fluticasone Propionate	
	2 h	14 h	26 h	14 h	26 h
PC ₂₀ AMP	2.18 (1.13–3.23)†	1.54 (0.48–2.59)†	1.3 (0.26 – 2.34)†	1.77 (0.7–2.75)†	0.33 (-0.69–1.34)
eNO	1.05 (0.9–1.23)	1.07 (0.91–1.25)	0.89 (0.77–1.04)	0.94 (0.81–1.09)	0.90 (0.78–1.04)

*Data for PC₂₀ AMP are expressed as doubling dose difference with 95% confidence interval between either FF or FP and Placebo.

Data for eNO are expressed as ratio (FF/placebo or FP/placebo) with 95% confidence interval.

†*P* < 0.05.

headache, nausea, palpitations and fatigue. None of the adverse events occurred more frequently during treatment with FF when compared to FP or placebo. Most of the adverse events were temporally associated with the AMP challenge procedure (dyspnea, dizziness, headache, nausea, palpitations and fatigue). Nine adverse events were considered to be related to the study medication by the investigator (either FP or placebo, no adverse events were judged to be related to FF). Sixteen subjects received concomitant medication (salbutamol) for either an adverse event (bronchoconstriction, dyspnea) or as rescue medication during the study.

Discussion

We present the first study investigating the efficacy and duration of action of a single dose of FF in the treatment of asthma. Both a single-dose FF and FP showed a significant improvement in the PC₂₀ AMP at 14 h. In addition, a single-dose FF, but not FP significantly improved the PC₂₀ AMP up to 26 h.

This study was not designed to directly compare both active treatments. For this reason, we only analysed the difference between the effects of FF and FP against placebo. Nevertheless, our findings suggest that FF may have a longer duration of action than FP, and this is compatible with earlier results from *in vitro* studies. For example, Salter et al. (8) investigated the pharmacological properties of different corticosteroids (FF, FP, mometasone furoate and budesonide) in cultured human epithelial cells. When adding the different corticosteroids to the apical site of an epithelial cell monolayer, FF was more rapidly absorbed and reached a higher maximal level in these cells than FP, mometasone furoate and budesonide. Salter et al. also demonstrated a slower rate of transport of FF out of epithelial cells than that seen with the other corticosteroids. In addition, Valotis et al. (11) have investigated receptor binding kinetics for FF in human lung tissue resection material obtained from patients with bronchial carcinomas. They showed that while FF has a statistically significantly higher association rate constant for binding to the human lung glucocorticoid receptor than FP or mometasone furoate (MF), it exhibits a dissociation rate constant similar to FP and MF. This resulted in calculated relative receptor affinity values of 2989 ± 135 for FF, 1775 ± 130 for FP ($P < 0.001$ vs FF) and 2244 ± 142 for MF ($P \leq 0.05$ vs FF). They also showed that FF is retained in human lung tissue to a greater extent than MF, but not FP. Taken together, these observations in "*in vitro*" studies might contribute to our clinical findings of a prolonged duration of action of FF in asthma patients. As FF exerts a significant protective effect against the PC₂₀ AMP up to 26 h, FF may be suitable for once-daily dosing in asthma. If this is the case, it could have important clinical consequences, because once-daily dosing may improve patient compliance which is generally poor in asthma patients (12). Since the effect of a single dose of either FP or FF was investigated, it was decided to use a relatively high dose of 1000 µg. It could

be argued that our results may have been different with lower doses. For this reason, it will be interesting to investigate the efficacy and duration of action of different doses of FF in future studies.

We investigated the protective effect of FF and FP against the PC₂₀ AMP at different time points after administration of a single dose. This model has previously been used to investigate both the efficacy and the duration of action of ICS. First, Ketchell et al. (6) found a dose-dependent protective effect after 2 h using FP 100, 250, or 1000 µg. In addition, we found a small improvement in the PC₂₀ AMP up to 26 h after administration of a single-dose FP of 1000 µg in a previous study (7). The latter may contrast with our present findings, because we did not find any protective effect of single dose of 1000 µg FP after 26 h. A possible explanation for this difference might be that the variability of the change in PC₂₀ AMP 26 h after treatment with FP was larger in this study and this may have decreased the power to detect an improvement, even though we included slightly more patients (18 patients completed this study compared to 13 in the previous study).

The concentration of eNO did not decrease after a single dose of FF or FP. There are two possible explanations for this finding: either the dose was not high enough or the treatment duration was not long enough. Thus far, only one study has found a decrease in eNO after a single-dose ICS (13). However, the dosage used in that study was very high (8000 µg) and the clinical relevance of such a high dose on eNO is questionable. Other studies with single-dose ICS were unable to find an effect on eNO. For example, Luijk et al. did not find a decrease in eNO at 2, 14, and 26 h after a single-dose FP 1000 µg, whereas the PC₂₀ AMP improved at all time points (7). Furthermore, Erin et al. (14) have observed a rapid (within 2.5 h) improvement in PC₂₀ AMP after the start of two different doses of ciclesonide (320 µg once daily and 640 µg b.i.d.), whereas a decrease in eNO was not observed until 3 days of treatment. Taken together, eNO, in contrast to the PC₂₀ AMP, does not change after single-dose ICS unless an extremely high dose is being administered.

Finally, short-term treatment with FF was found to be safe and well tolerated. We did not find any serious adverse events after inhalation of FF and the number of mild adverse events was not higher after treatment with FF compared with FP or placebo.

In conclusion, we have shown that the newly developed ICS, FF, protects against the PC₂₀ AMP up to 26 h after administration of a single dose. Our findings may suggest a longer duration of action for FF, since no protective effect was observed 26 h after administration of a single-dose FP. Whether this is really the case has to be confirmed in future studies. This is important, because it may enable once-daily dosing in asthma patients and improve patient compliance.

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