An assessment of the systemic activity of single doses of inhaled fluticasone propionate in healthy volunteers

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- 1 The systemic effects of inhaled fluticasone propionate (FP), administered via Diskhaler®, on the hypothalamo-pituitary-adrenal (HPA) axis were assessed primarily by measuring plasma cortisol at frequent intervals for 20 h after drug administration.
- 2 FP showed a dose-related suppression of plasma cortisol measured as area under the plasma cortisol vs time curve (AUC 0–20). The cortisol suppression (expressed as % fall from placebo) was 8, 19, and 28% for single doses of 250 µg FP, 500 µg FP and 1000 µg FP, respectively. A single dose of budesonide, 800 µg (via Turbuhaler®), resulted in a 16% cortisol suppression. The cortisol suppression for all three single doses of FP, and for the single dose of budesonide, was statistically significantly different from placebo.
- 3 Repeated dosing of FP (1000 µg twice daily for 3.5 days) resulted in a more marked plasma cortisol suppression; a fall of 65% from placebo (AUC FP 1000 mg twice daily vs AUC placebo, $P < 0.001$).
- 4 In a well-controlled study in healthy volunteers, inhaled FP, in therapeutic doses, was shown to exhibit systemic effects which appear to be more pronounced after repeated dosing.

Keywords plasma cortisol urine cortisol suppression fluticasone propionate

Introduction

Fluticasone propionate (FP) is a recently developed topical glucocorticosteroid (GCS) intended for the treatment of asthma and rhinitis. It has been claimed that inhaled FP, in therapeutic doses, lacks significant systemic effects. This claim is based primarily on mean, single morning plasma cortisol concentrations [1, 2]. However, it has been shown [3] that cortisol concentrations measured in single morning samples can vary greatly, thus limiting their value as an indicator of a GCS effect on the hypothalamo-pituitary-adrenal (HPA) axis. In a placebo-controlled study in healthy subjects, in which the effect of different blood sampling regimens was examined, Jennings *et al.* [4] showed that multiple blood sampling at frequent intervals gave a more complete and reproducible measure of GCS systemic activity. There is no published data showing the effect of inhaled FP on the HPA-axis using the more sensitive multiple sampling method.

FP is a highly potent, topical GCS which is eliminated primarily via hepatic clearance [5]. Since inhaled FP is absorbed from the lung to the systemic

circulation [6], the suggested lack of systemic effect, even in doses as large as 2000 µg daily, is somewhat surprising. The primary aim of this study was to assess the systemic activity, measured as plasma cortisol suppression, of three single doses of inhaled FP as compared with placebo, utilizing a sensitive multiple sampling technique. A single dose of budesonide was included as a reference. The effect of the highest recommended dose of FP (1000 µg twice daily) was also examined after repeated dosing for 3.5 days (7 doses).

Methods

Subjects

Twenty-five (25) non-smoking, healthy, male volunteers entered and completed the study. Their mean age was 24 years (range 18–30 years), their mean height, 181 cm (165–197 cm) and their mean weight,

76 kg (65–93 kg). The subjects were fully informed about the purpose of the study and the investigational events and possible risks involved in the study. Each subject gave his written consent before inclusion into the study. The study was approved by the Medical Ethics Committee of Uppsala University and by the Swedish Medical Products Agency (Uppsala).

Study design

The study comprised two parts, with the same subjects participating in both parts:

Part 1 was performed with an open, randomized (according to Williams), balanced cross-over, placebo-controlled design.

Single doses of 250 µg, 500 µg and 1000 µg fluticasone propionate (Flixotide® Diskhaler®, Allan & Hanbury) were compared with placebo Turbuhaler® and 800 µg budesonide (Pulmicort® Turbuhaler®, Astra). All single doses were administered at 22.00 h. There was a washout period of at least 4 days between any two consecutive treatments. During each study period at the laboratory, blood samples for cortisol analysis were collected at regular intervals. A 24 h urine sample, for analysis of cortisol, was also collected.

Part 2 was performed with an open, non-randomized, multiple dose design. Following completion of the randomized part of the study (Part 1) and a washout period of at least 4 days, all subjects received fluticasone propionate (Flixotide® Diskhaler®) 1000 µg twice daily for 3.5 days (7 doses). The first dose was given at 22.00 h on day 0 and the last dose (7th) at 22.00 h on day 3. The dosing interval was 12 h. Following the 7th dose, blood samples for analysis of cortisol were collected as after each of the single doses. A 24 h urine sample was also collected.

Samples of the batches of each of the study drugs were analyzed for drug content. Of the nominal dose of FP 250 µg, the average blister content was 220 µg. Of the nominal dose of 400 µg budesonide, the average metered dose was 380 µg.

Investigational procedures

On the investigational days (Parts 1 and 2), a venous catheter was inserted into a forearm vein. Inhalation of each of the single doses and the 1st and 7th of the multiple doses was performed at the clinic under the supervision of a staff member. The subjects were instructed in, and used, the inhalation technique given in the package insert for each of the drugs/devices. After inhalation, the subjects rinsed their mouth and spat out the water. Mouth-rinsing was also performed after each of the multiple doses at home. Blood samples (5 ml) for cortisol analysis were taken at the following time points: 0, 2, 4, 6, 8, 10, 12, 14, 17 and 20 h after study drug administration at the clinic. The samples were taken in heparinized tubes and centrifuged at 3000 rev min⁻¹ at +20°C for 10 min. The plasma was decanted and stored at -20°C until analysed. Urine (for analysis of cortisol) was collected in one 24 h fraction starting at 22.00 h on

study days at the clinic. All subjects emptied their bladder immediately before administration of study drugs. The urine was collected in pre-weighed polyethylene bottles and, during the 24 h collection period, was stored in a refrigerator at +4°C. On completion of the urine sampling, the bottles were reweighed and the urine volume was calculated assuming a density of 1.02 g ml⁻¹. For each subject, the urine was carefully mixed and, thereafter, two 10 ml samples were transferred to polystyrene test tubes and stored at -20°C until analysed.

Bioanalytical methods

The analysis of blood and urine samples for cortisol was performed blindly.

The analysis of plasma cortisol was performed at the Department of Clinical Chemistry, University Hospital, Uppsala. The plasma concentrations of cortisol were determined by radioimmunoassay (ORION DIAGNOSTICA Cortisol [¹²⁵I]). The assay had a minimum determinable concentration (MDC) of 3–6 nmol l⁻¹ with an inter- and intra-assay coefficient of variation ≤ 9%. All samples (including standard curves) for one subject were analyzed in one batch. This assay has been validated against GC-MS.

The analysis of urine cortisol was performed at Astra Draco AB, Lund, Department of Bioanalytical Chemistry. The cortisol concentrations were determined by an LC-thermospray-MS method [7, 8]. The assay had a minimum determinable concentration (MDC) of 6 nmol l⁻¹ with a within-day coefficient of variation of 3–7%.

Data analysis

Plasma cortisol was examined by calculating area under the plasma cortisol concentration vs time curve for the 20 h sampling interval (AUC 0–20). The area was calculated according to the linear trapezoidal method. If the actual sampling time deviated by more than 5% from the stipulated time, the actual time was utilized.

Summary statistics (including arithmetic means, medians, standard deviation, standard error of the mean and % coefficient of variation) were calculated. Treatment differences were assessed using ANOVA (SAS GLM Procedures). Analyses were performed on log-transformed data. Ninety-five per cent confidence intervals were utilized for the comparisons between treatments/doses. For Part 1 of the study, a full ANOVA model was utilized (subject[sequence], treatment, period, sequence and error). For Part 2, a reduced ANOVA model (subject, treatment, error) was used.

Results

The time courses of changes in plasma cortisol following study drug administration are shown in Figure 1.

Figure 1 Mean plasma cortisol profiles during the 20 h period following single doses of placebo (□), FP 250 µg (●), 500 µg (○), 1000 µg (◇), and budesonide 800 µg (◆) and following the last of seven doses of FP 1000 µg twice daily (■).

Table 1 Plasma cortisol (AUC 0–20) and urine cortisol (24 h) values (means and 95% confidence intervals) for each of the study treatments.

	Placebo		Fluticasone		Budesonide	
	single	250 µg single	500 µg single	1000 µg single	1000 µg twice daily	800 µg single
AUC 0–20 plasma cortisol (nmol l ⁻¹ h)	4411	4083	3565	3196	1548	36788
95% CI	4200–4631	3846–4240	3395–3744	3044–3356	1337–1792	3503–3862
24 h urine cortisol (nmol)	50.2	50.3	44.2	38.5	15.5	44.4
95% CI	42.4–59.4	42.6–59.3	38.7–50.4	32.3–45.9	11.0–21.9	38.1–51.7

All of the active study drugs resulted in a cortisol suppression as compared with placebo. The cortisol suppression was most marked for the multiple dose treatment with FP 1000 µg twice daily.

The mean AUC 0–20 value (LSmean) and 95% confidence intervals for each of the treatments is shown in Table 1. The cortisol suppression AUC 0–20, expressed as a percent fall from placebo, is shown in Figure 2.

For single doses of fluticasone propionate, there was a statistically significant dose-related suppression of cortisol, measured as AUC 0–20. The multiple dose treatment with 1000 µg twice daily, FP resulted in a marked suppression from placebo, which, at 65%, was more than double that seen with a single dose of 1000 µg (28%). The single dose of budesonide (800 µg) resulted in a 16% suppression of plasma cortisol and was also statistically significant as compared with placebo.

Figure 2 Mean plasma cortisol suppression, measured as AUC 0–20 and expressed as percent fall from placebo (based on LSmean), for single doses of budesonide 800 µg and FP 250 µg, 500 µg, 1000 µg, and following the last of 7 doses of FP 1000 µg twice daily. The asterisks represent significance levels vs placebo: * $P < 0.05$, *** $P < 0.001$.

Figure 3 Individual morning (08.00 h) plasma cortisol concentrations for the 25 subjects participating in the study following single doses of budesonide 800 µg, placebo and FP 250 µg, 500 µg, 1000 µg, and following the last of seven doses of FP 1000 µg twice daily. Mean values are indicated by a bar.

The distribution of morning plasma cortisol (08.00 h) values with the five single dose treatments and the multiple dose FP treatment is shown in Figure 3.

Urine cortisol analysis revealed the same pattern as for plasma cortisol i.e. single doses of fluticasone showed a dose-related suppression of cortisol and the multiple dose treatment resulted in a more marked cortisol suppression. The mean 24 h urine cortisol values (nmol) and the 95% confidence intervals are shown in Table 1.

Discussion

Assessment of the effect on plasma cortisol is an accepted measure of the systemic activity of GCS. With low to medium doses of inhaled, topical steroids, such as budesonide and beclomethasone dipropionate, suppression of the HPA axis does not appear to present a problem of clinical relevance *per se*. With higher daily doses (> 1500 µg), the HPA axis does appear to be affected [9]. The safety of inhaled GCS has recently been reviewed [10]. At doses of ≤ 1500 µg daily, most studies have not found any impairment of the short ACTH response in adults and no cases of clinical adrenal insufficiency have been reported in patients treated with inhaled steroids only. However, suppression of the HPA-axis indicates that the steroid being tested is systemically available and active. There is still some clinical concern [10] regarding the possible adverse effects of systemically active GCS during long-term treatment with inhaled GCS, but it should be noted that effects on the HPA-axis may not necessarily predict other systemic effects.

The results of the present study show that even after single administration, and with a dose as low

as 250 µg, fluticasone propionate is present in the systemic circulation in sufficient amounts to affect the HPA axis. In the present study, mouth rinsing was employed to minimize the amount of orally deposited drug. However, since FP is claimed to have virtually zero bioavailability [5], the contribution from any swallowed FP to the systemic effect would also be virtually zero. Thus, the systemic activity seen with FP in the present study must arise from substance which has been absorbed via the lung.

It is interesting to note that repeated dosing with high dose FP for only 3.5 days (7 doses) resulted in a more marked cortisol suppression as compared with a single dose. The explanation for the more marked cortisol suppression is unclear. It has recently been claimed [6] that the terminal $t_{1/2}$ of inhaled FP is approximately 4 h. With a $t_{1/2}$ of 4 h and a dosing interval of 12 h, it is unlikely that accumulation of FP in blood or plasma could account for the marked increase in cortisol suppression observed in the present study.

Since only one dose level of budesonide, the active reference, was given in this study, a systemic potency relationship between FP and budesonide cannot be calculated. However, the cortisol suppressive effect of a single dose of FP 500 µg was equivalent to the suppressive effect of a single dose of budesonide 800 µg. This would suggest that the systemic effect of FP is greater than that of budesonide, despite the fact that lung deposition of FP via Diskhaler® is less than that of budesonide via Turbuhaler® (12% [11] and 27% [12] respectively) and despite the fact that there is virtually no contribution to the systemic effect of orally deposited drug due to almost complete first-pass metabolism of FP [5]. However, FP does have a longer $t_{1/2}$ (4 h [6]) than budesonide ($t_{1/2}$ 2.0 h [13]).

These results in normal subjects show a degree of cortisol suppression which has not previously been

reported in asthmatic patients receiving FP. A difference in the proportion of central vs peripheral drug deposition in the lung between asthmatics and normals cannot be excluded. However, it is unclear if such a difference could explain alterations in the degree or rate of absorption of the drug. In addition, in a study in patients receiving 1500 µg FP for 1 year [14], 25 of 110 patients (23%) showed a fall in 24 h urinary free cortisol from within to below normal limits, indicating that systemic activity of FP can be observed even in asthmatic patients.

The current study is a human pharmacological study performed under very stringent conditions. During blood and urine sampling periods, the subjects were under observation at the clinical

research unit. The time point for study drug administration was pre-set to 22.00 h and all blood sampling times and the urine collection period were closely monitored. Thus, inter-subject variations, with respect to sampling periods, were minimized. These stringent conditions are not usually met in a clinical study, and thus variations in study drug administration time, blood sampling times and urine collection periods may mask effects of the drug on, for example, cortisol suppression and may explain the apparent absence of systemic effects in some clinical studies [1, 2].

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