Effects of oral and inhaled corticosteroid on lymphocyte β_2 -adrenoceptor function in asthmatic patients

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Aims We have previously demonstrated that a single dose of oral prednisolone but not single doses of inhaled fluticasone had facilitatory effects on lymphocyte β_2 -adrenoceptor (AR) function. To address possible differences in steady-state time-course, the aim of this study was to determine if repeated dosing with inhaled fluticasone would have facilitatory effects on lymphocyte β_2 -AR. Plasma cortisol was also evaluated as a measure of systemic bioactivity.

Methods Ten asthmatic subjects, mean (s.e.mean) age 29 (3) years, FEV₁ 89 (5) % predicted, were randomised in a double-blind crossover study to receive inhaled placebo (PL), inhaled fluticasone 1000 $\mu\text{g day}^{-1}$ (F1000) and inhaled fluticasone 2000 $\mu\text{g day}^{-1}$, each for 4 days and also a single dose of oral prednisolone 50 mg (PRED). Prednisolone was given as open medication. The last dose of study drug was taken at 22.00 h and subjects attended the laboratory at 08.00 h the following day.

Results β_2 -AR density (B_{max} ; fmol/ 10^6 cells) was significantly increased after PRED compared with PL and inhaled fluticasone. B_{max} (geometric mean) after each treatment were: PL 1.51, F1000 1.20, F2000 1.20 and PRED 2.14 (a 1.4 fold difference PRED *vs* PL; 95% CI 1.05 to 1.95; $P < 0.001$). There was significant ($P < 0.001$) suppression of plasma cortisol (nmol l^{-1}) following F2000 and PRED compared with PL: 393.8, F1000 302.1, F2000 205.0 (95% CI F2000 *vs* PL 58.1 to 319.4) and PRED 87.0 (95% CI PRED *vs* PL 176.2 to 437.5). The estimated milligram equivalence ratio for adrenal suppression was calculated at 1:11 for fluticasone *vs* prednisolone.

Conclusions Repeated dosing with high-dose inhaled fluticasone did not up-regulate lymphocyte β_2 -AR as compared with a single dose of oral prednisolone, despite having significantly suppressed early morning plasma cortisol. This study confirms our previous finding of a dissociation in sensitivity between effects of inhaled corticosteroid on adrenal suppression and lymphocyte β_2 -AR regulation, at least for doses up to 2 mg day^{-1} of fluticasone.

Keywords: fluticasone, prednisolone, lymphocyte β_2 -adrenoceptors, asthma

Introduction

Corticosteroids are the most effective therapy in asthma, and inhaled corticosteroids form the cornerstone of anti-asthma treatment at present [1, 2]. Inhaled corticosteroids are now considered appropriate therapy for patients who need inhalation of β_2 -adrenoceptor agonist more than once daily [1]. Although the anti-inflammatory actions of inhaled corticosteroid are well known [3–6], the facilitatory effects on β_2 -adrenoceptor (AR) function is less clear. Systemic corticosteroids have been shown to reverse β_2 -AR down-regulation in normal subjects [7, 8] and asthmatics [9] who have been exposed to β_2 -adrenoceptor agonists. Studies in asthmatic patients receiving regular long-acting β_2 -adrenoceptor agonists have shown that β_2 -AR down-regulation and associated bronchodilator subsensitivity occur despite the use of inhaled corticosteroid [10–12]. We have previously

demonstrated that single inhaled doses of fluticasone propionate (1000 μg and 2000 μg) did not have any significant facilitatory effects on lymphocyte β_2 -AR in asthmatic patients, in contrast to oral prednisolone, which produced up-regulation [13]. This begs the question as to whether this lack of effect with single doses of fluticasone may be due to pharmacokinetic properties and hence its time-profile for up-regulation.

To address possible differences between single dose and steady-state time-profiles, we have performed a further study to see whether up-regulation might occur at steady-state with repeated dosing of fluticasone propionate.

Methods

Patients

Ten asthmatic subjects (five female), mean age 29 years (range 19–52) were recruited to completion in this study. All gave written informed consent before being randomized

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into a double-blind, placebo controlled, cross-over study which was approved by the Tayside Committee on Medical Research Ethics. A full physical examination, haematological and biochemical profile were normal prior to inclusion into the study. All had asthma according to the criteria of the American Thoracic Society [14] and all were non-smokers. Mean (s.e.mean) FEV₁ in litres and % predicted were 3.09(0.09) l and 89(5)% predicted, range 2.70 to 3.54 (% predicted range 65–108%). Nine subjects were inhaling corticosteroid (either budesonide or beclomethasone dipropionate) in a median dose of 400 µg day⁻¹, range 200 to 800 µg day⁻¹. All were inhaling a short-acting β₂-adrenoceptor agonist as required in doses of <400 µg day⁻¹. None had received oral corticosteroid for at least 3 months, and none had had a recent exacerbation of asthma in the past month.

Protocol

All usual inhaled corticosteroids were discontinued 4 days prior to the study and for the duration of the study. Subjects were randomized to receive either inhaled placebo, inhaled fluticasone 1000 µg day⁻¹ (given as 500 µg twice daily; Flixotide, Allen & Hanburys, Uxbridge, UK), inhaled fluticasone 2000 µg day⁻¹ (given as 1000 µg twice daily) and oral prednisolone 50 mg. All inhaled medication was delivered by a metered dose inhaler, which was taken at 08.00 h and 22.00 h with mouth rinsing after each dose. The study medication was taken for 4 days with a 7-day washout between each treatment. Subjects attended the laboratory at 08.00 h, with the last dose of study drug taken at 22.00 h the previous night. Patients were instructed in the use of a metered-dose inhaler with the help of an aerosol inhalation monitor (Vitalograph Ltd, Buckingham, UK).

Oral prednisolone was given as open-medication but within the randomization scheme. 50 mg of oral prednisolone was taken at 22.00 h and the subjects attended the laboratory at 08.00 h the following day. At each visit, an intravenous cannula was inserted into an antecubital vein. After 15 min supine rest, 50 ml of blood was withdrawn for plasma cortisol and lymphocyte β₂-AR parameters (B_{max}, E_{max} and K_d).

Measurements

Lymphocyte β₂-adrenoceptor function Lymphocyte β₂-AR function was measured as previously described [10]. In brief, 40 ml whole blood was collected into tubes containing ethylenediamine tetra-acetic acid, diluted to 50 ml with phosphate buffered saline (PBS), and then two equal aliquots were centrifuged with 15 ml of lymphoprep (Nycomed Pharma AS, Oslo, Norway) and the lymphocyte layer subsequently removed. Following two further washes with PBS and centrifugation, the lymphocyte pellet was resuspended in 5 ml of PBS prior to lymphocyte counting, with 5 × 10⁶ cells being required for cyclic AMP stimulation by isoprenaline. The suspension was re-centrifuged and the lymphocyte pellet resuspended in lymphocyte resuspension buffer to give a concentration of 5 × 10⁶ cells/ml. β₂-AR density (B_{max}) and binding affinity (K_d) were assayed by

incubating the suspension for 1 h at 37° C with (–)¹²⁵I-iodocyanopindolol (ICYP, NEN-du Pont Ltd, Stevenage, UK) at concentrations of between 5 and 160 pm. Half of the tubes contained CGP 12177 (Ciba-Geigy, Basel, Switzerland) to prevent ICYP binding to β₂-receptors. After washing with assay buffer, the suspensions were aspirated onto filter paper using a Brandel Cell Harvester (Semat Technicare Ltd, St Albans, UK) and the resultant counts determined by a gamma camera (LKB Wallac, Wallac OY Pharmacia, Turku, Finland). Specific (receptor) binding was calculated from total binding minus non-specific (non-receptor) binding. Receptor density was calculated from extrapolation from the plateau of the specific binding curve plotted for each concentration of ICYP by curve-fitting analysis. Receptor affinity was calculated from the concentration of radioligand required to produce 50% of maximal specific binding. The intra-assay co-efficient of variation for analytical imprecision was 5.9% for K_d and 10.3% for B_{max}. The suspension containing 5 × 10⁶ cells was centrifuged and the pellet resuspended in PBS containing theophylline (100 µg) and bovine serum albumin. It was stimulated with isoprenaline (10–4 M) during incubation at 37° C, before terminating the reaction by heating to 95° C. After centrifugation, the supernatant was removed and cAMP determined by radioimmunoassay (Incstar Ltd, Wokingham, UK) with the intraassay and interassay coefficients of variation for analytical imprecision being 5.36% and 9.3% respectively.

Plasma cortisol Samples for plasma cortisol were stored at –20° C and analysed at the end of the study. Plasma cortisol was analysed by radioimmunoassay (Sorin Biomedica, Saluggia, Italy). The intra- and inter-assay coefficients of variation for analytical imprecision were 4.5% and 8.1% respectively.

Statistical analysis Data for B_{max} were log-transformed prior to analysis as it is not normally distributed. For all parameters, comparison between treatments was by multifactorial analysis of variance (MANOVA) with subjects and treatments as within factors for analysis. Where significant differences occurred by MANOVA, Bonferroni multiple-range testing was applied to identify where these differences occurred. A probability value of *P* < 0.05 (two-tailed) was considered as being of significance and 95% confidence intervals (95% CI) for mean treatment differences were calculated where significant. Data were analysed using a Statgraphics Statistical Software package (STSC Software Publishing Group, Rockville, USA). The milligram equivalence ratio for fluticasone *vs* prednisolone was estimated by log-linear extrapolation.

Results

Lymphocyte β₂-AR parameters

β₂-AR density (B_{max}; fmol/10⁶ cells) was significantly increased after oral prednisolone but not after inhaled fluticasone. The geometric mean values for B_{max} being as follows: PL 1.51, F₁₀₀₀ 1.20, F₂₀₀₀ 1.20 and PRED 2.14 (a 1.4 fold difference PRED *vs* PL; 95% CI 1.05 to 1.95;

$P < 0.001$) (Figure 1). There was no significant difference in K_d following any of the treatments. (Table 1). $E_{\max}$ showed a similar trend to effects observed with $B_{\max}$ but was not significantly different.

Plasma cortisol There was significant ($P < 0.001$) suppression of plasma cortisol (nmol l^{-1}) following fluticasone 2000 μg (F_{2000}) and oral prednisolone compared with placebo (PL): PL 393.8, F_{1000} 302.1, F_{2000} 205.0 (95% CI F_{2000} *vs* PL 58.1 to 319.4) and prednisolone 87.0 (95% CI prednisolone *vs* PL 176.2 to 437.5). There was suppression of plasma cortisol following F_{1000} . (Figure 1) which was not significant. The estimated milligram equivalence ratio for adrenal

suppression was calculated at 1:11 for fluticasone *vs* prednisolone.

Discussion

The results of this study shows that repeated dosing with high dose inhaled fluticasone did not have any facilitatory effects on lymphocyte β_2 -AR despite having significantly suppressed early morning plasma cortisol with an estimated milligram equivalence ratio of 1:11 *vs* prednisolone. This result is in contrast to that with a single dose of oral prednisolone which, although suppressing plasma cortisol, significantly up-regulated β_2 -AR density. This study confirms our previous finding that single doses of inhaled fluticasone did not up-regulate β_2 -AR [13]. Both of these studies suggest a dissociation in sensitivity between effects of inhaled corticosteroid on adrenal suppression and lymphocyte β_2 -AR regulation. Our patients had relatively mild disease and had been exposed to small amounts of β_2 -adrenoceptor agonists, mostly on an as required basis. Thus, it is conceivable that their β_2 -AR were not down-regulated prior to the study, explaining the lack of up-regulation with inhaled corticosteroid. This, of course, does not explain why prednisolone produced up-regulation of apparently naive β_2 -AR, as was the case in our previous study [13]. It may be that higher doses of fluticasone (i.e. > 2 mg) may up-regulate β_2 -AR, although this would not be clinically relevant as the highest licensed dose is 2 mg day^{-1} . Conversely, it would also be interesting to know whether up-regulation occurs with lower doses of prednisolone (e.g. 22 mg) with approximately equivalent adrenal suppression to fluticasone 2 mg. Dose-ranging studies would be required to answer this important question.

We accept our study has its limitations as we used lymphocyte β_2 -adrenoceptors as a surrogate for lung β_2 -adrenoceptors, although there is some evidence to suggest that this may be valid [15]. One would expect that fluticasone given by the inhaled route would produce higher local concentrations in the airway than in the systemic compartment. Nonetheless, inhaled fluticasone (Diskhaler) is well absorbed with 16% bioavailability [16]. Indeed, we showed evidence of systemic absorption in terms of adrenal suppression with an 11:1 mg ratio *vs* oral prednisolone. Further studies are required to look at possible differential effects of inhaled and oral corticosteroids on airway β_2 -adrenoceptor function in asthmatic patients, in terms of bronchoprotective or bronchodilator effects of regular β_2 -adrenoceptor agonist treatment.

Systemic corticosteroids, as well as having potent anti-inflammatory effects, have facilitatory effects on the β_2 -AR. Corticosteroids regulate the number of β_2 -AR [17] and the coupling of the receptor to the adenylate cyclase system

Figure 1 Values are shown for lymphocyte β_2 -receptor binding density ($\log B_{\max}$) and plasma cortisol after treatment with inhaled placebo (PL), inhaled fluticasone 1000 $\mu\text{g day}^{-1}$ (F_{1000}), inhaled fluticasone 2000 $\mu\text{g day}^{-1}$ (F_{2000}), each given for 4 days, and after a single dose of oral prednisolone 50 mg day^{-1} (PRED). Values are mean ($\pm$ s.e. mean). (* denotes $P < 0.05$ *vs* PL).

Table 1 Receptor binding affinity (K_d) and maximal cyclic AMP response to isoprenaline ($E_{\max}$) after treatment with inhaled placebo (PL), inhaled fluticasone 1000 $\mu\text{g day}^{-1}$ (F_{1000}), inhaled fluticasone 2000 $\mu\text{g day}^{-1}$ (F_{2000}) and oral prednisolone 50 mg day^{-1} (PRED). Values are mean and 95% CI.

	PL	F1000	F2000	PRED
K_d	16.3	12.2	14.8	13.2
(pmol l^{-1})	(11.7–20.9)	(7.3–17.1)	(10.2–19.4)	(8.6–17.8)
$E_{\max}$	4.85	4.64	4.39	5.91
($\text{pmol}/10^6$ cells)	(3.72–5.97)	(3.52–5.77)	(3.26–5.51)	(4.78–7.03)

[18]. Receptor regulation by steroids is probably by a process of increased β_2 -AR gene transcription [19]. Likewise, systemic corticosteroids demonstrate significant facilitatory effects on β_2 -AR by reversing β_2 -AR down-regulation [6–9]. Inhaled corticosteroids also demonstrate potent anti-inflammatory activity [3–6]. However, our studies have demonstrated that inhaled high-dose corticosteroids do not appear to have facilitatory effects on the β_2 -AR. In this respect, inhaled corticosteroid therefore would not be expected to protect against β_2 -AR down-regulation and tachyphylaxis. This has been demonstrated in several studies involving formoterol [10, 11] and salmeterol [12], where β_2 -AR down-regulation and bronchodilator subsensitivity developed despite the fact that most of the patients were receiving inhaled corticosteroid.

In summary, this study has shown that repeated dosing with high-dose inhaled fluticasone had no significant facilitatory effects on lymphocyte β_2 -adrenoceptor function in asthmatic patients, despite suppressing adrenal function with an estimated milligram equivalence ratio of 1:11 compared with prednisolone.

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