EFFECTS OF CICLESONIDE AND FLUTICASONE ON CORTISOL SECRETION IN PATIENTS WITH PERSISTENT ASTHMA

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ABSTRACT

We compared the systemic and clinical effects of ciclesonide (CIC) and fluticasone propionate (FP), given on top of CIC 160 µg/day and salmeterol 50 µg twice daily in 32 patients with persistent asthma using a randomized double-blind, placebo-controlled, double-dummy, five-period crossover design.

All patients exhibited a PC$_{20}$ methacholine <8 mg/ml and a PC$_{20}$ adenosine <60 mg/ml. Primary outcome was 24-h serum cortisol suppression after seven days. Secondary outcomes were changes in PC$_{20}$ methacholine and adenosine after 9 days.

FP 500 µg/day and 1000 µg/day significantly suppressed cortisol secretion versus placebo by –46.2 (95%C.I.: –83.8,–8.5) nmol/L and by –76.1 (95%C.I.: –112.9,–39.3) nmol/L, respectively. Neither dose of CIC (320 or 640 µg/day) had a significant suppressive effect [–28.2 (95%C.I.: –65.5,+9.2) nmol/L and – 37.3 (95%C.I.: –74.7, 0.0) nmol/L, respectively]; differences between FP 1000 µg/day and both CIC treatments were statistically significant [for CIC 320 µg/day: –48.0 (95%C.I.: –84.8,–11.1) nmol/L; for CIC 640 µg/day: –38.8 (95%C.I.: –75.7,–1.9) nmol/L]. Compared with placebo, the increase in PC$_{20}$ adenosine after the four treatments was small, but significant. Greater improvements in PC$_{20}$ adenosine were seen with FP 500 µg/day [1.8 (95%C.I.: 1.0, 2.6) doubling concentrations] compared with CIC 320 µg/day [0.9 (95%C.I.: 0.1, 1.7) doubling concentrations]; no significant difference was seen between CIC 640 µg/day and FP 1000 µg/day.

For a similar decrease in hyperresponsiveness, cortisol secretion was suppressed significantly with moderate to high doses of FP, but not with CIC.

Key words: aerosol therapy, anti-asthmatic agent, asthma, bronchial hyperreactivity, inhaled corticosteroids, cortisol
INTRODUCTION

Inhaled corticosteroids (ICS) are the most effective controller medications currently available to treat asthma. They reduce airway inflammation and hyperresponsiveness, improve symptoms, pulmonary function and quality of life [1,2] and decrease hospitalizations [3] and mortality rate [4]. ICS are thus the guideline-recommended first-line treatment for all patients with persistent forms of the disease [5]. Although the vital role of ICS in the management of asthma is generally recognized and ICS are well tolerated at low-to-medium doses, it has been claimed that the long-term administration of high doses of ICS has a potential for systemic adverse events (AEs), such as growth inhibition, osteoporosis, suppression of hypothalamic–pituitary–adrenal (HPA)-axis function [6] or even adrenal crisis [7]. This potential for AEs with ICS is a concern for patients and physicians, and may contribute to intentional nonadherence [8] and sub-optimal prescribing [9].

Ciclesonide (CIC) is a novel, airways-targeted ICS that is delivered as an inactive compound and converted by esterases to the active metabolite (desisobutyryl-ciclesonide) in the airways, where it elicits its anti-asthmatic effect [10-13]. Several trials have shown that doses up to 1280 µg/day of CIC do not produce clinically relevant HPA-axis suppression in both healthy volunteers and asthma patients [13-18]. Fluticasone propionate (FP) is an established ICS, which has, however, been associated with pronounced suppression of HPA-axis function in healthy volunteers [19] and to a lesser extent, in asthma patients [14,15,20-22]. The current study was thus designed to assess the safety of CIC and FP in patients with persistent asthma chronically treated with ICS. More specifically, we wanted to address: 1) whether moderate-to-high doses of inhaled CIC suppress 24-h serum and urinary cortisol levels and biochemical markers of bone formation in patients with moderate persistent asthma and how these effects compare with those of moderate-to-high doses of FP; 2) to what extent do moderate-to-high
doses of CIC reduce airway responsiveness to adenosine and methacholine, and how these
effects compare with those of moderate-to-high doses of FP; and 3) whether one of the two
investigated formulations is superior in terms of the ratio between clinical effect and systemic
effect.

METHODS

Patients
Male and female patients (aged 18–65 years) known to have persistent asthma for more than
6 months, as defined by the Global Initiative for Asthma GINA, were allowed to participate in
the study. Patients were included if their current treatment consisted of a constant dose of a
moderate to high daily dosage of ICS alone (beclomethasone dipropionate ≤1000 µg/day or
equivalent) or a combination of low doses of ICS with long-acting β₂-agonists (LABA)
(beclomethasone 200 µg twice daily [bid] or equivalent plus salmeterol 50 µg bid or
formoterol ≤12 µg bid) for more than 4 weeks. Patients with severe persistent asthma were
excluded to avoid drop-outs. Patients had to demonstrate a FEV₁ of >60% predicted at the
study start and at randomization. They all exhibited a PC₂₀ methacholine (provocative
concentration of inhaled methacholine leading to a 20% decrease in post-saline FEV₁) <8
mg/mL) and a PC₂₀ adenosine (provocative concentration of adenosine leading to a 20%
decrease in post-saline FEV₁) <60 mg/mL. Patients were also required to have normal HPA-
axis function (serum cortisol concentration at 8.00 am [± 30 minutes] >5 µg/dL [>138
nmol/L]) and not to have experienced an asthma exacerbation or respiratory tract infection
within 8 weeks prior to the start of the study.

Patients were excluded if they had used systemic steroids within 4 weeks of study start or
more than three times during the last 6 months; had chronic obstructive pulmonary disease
and/or other pulmonary diseases; had a history of other medical conditions known to affect
cortisol levels (e.g. Cushing’s Syndrome); or were receiving drugs known to affect endogenous cortisol production (e.g. anabolic steroids or androgens). Females were excluded if they were pregnant, giving breast feeding or not using safe contraception, were of childbearing potential, or were <1 year postmenopausal.

This study was conducted in accordance with the rules of the International Conference on Harmonization Good Clinical Practice and the ethical principles of the Declaration of Helsinki. Written consent was obtained from the patients before the start of the study, and the protocol was reviewed and approved by the appropriate Independent Ethics Committee or Institutional Review Boards.

**Study design**

This randomized, double-blind, double-dummy, placebo-controlled, five–period crossover study was conducted at two centers in Belgium (Fig. 1). During a 4–6 week run-in period, patients were administered CIC 160 µg in the evening plus salmeterol 50 µg bid. This treatment was continued throughout the entire study. Ciclesonide was chosen because studies have previously shown that daily doses of up to 1280 µg CIC had no clinically relevant effect on cortisol secretion [14,16,17]. Following the run-in period, patients were randomly assigned to one of ten treatment sequences, occurring in a Latin square and its mirror, for which a computer generated randomization list was used (Table 1). These ten sequences were uniform on the periods (each treatment was applied with the same frequency in each period) and on the subjects (each treatment was applied with the same frequency within each subject), and balanced with respect to a first order carry-over effect (each treatment preceded every other treatment a same number of times).

Each treatment sequence consisted of five-period treatments which contained one of the following study medications (all administered via hydrofluoroalkane metered dose inhaler
[HFA]–MDI) given on top of the CIC 160 µg/day maintenance dose: CIC 160 µg bid (ex-actuator); CIC 320 µg bid (ex-actuator); FP 250 µg bid (ex-valve; 220 µg bid ex-actuator); FP 500 µg bid (ex-valve; 440 µg bid ex-actuator); or placebo. Due to the code labeling, neither the investigator nor anyone at the study center knew which drug or dosage was administered. The FP doses were based on previous observations, showing equivalence of CIC 320 µg/day with FP 500 µg/day in terms of bronchial responsiveness to methacholine [15,23]. The study medication was inhaled at 8:00 am and 8:00 pm (± 30 min), starting at the evening of each period. The last inhalation took place 30–60 min before the methacholine provocation on the ninth day of treatment. Each treatment period was separated by a 4–12-week washout period, to allow for all previously administered study drug to be cleared from the system and to allow PC20 adenosine [14] and serum cortisol [20,24] to return to baseline values.

**Spirometry and measurement of airway hyperresponsiveness**

Spirometry was performed at study start (between 8.00–10.00 am) and repeated at the beginning and end of each 9-day treatment period at approximately the same time point. The highest value from three acceptable tests was recorded for FEV1. Rescue medication had to be withheld for ≥8 h and LABAs for ≥24 h prior to each lung function measurement. Challenge tests were performed at the study start and at the end of each treatment period (visits T2, T5, T8, T11 and T14; Fig. 1) 30–60 min after the last dose of study medication, according to a protocol that has been described previously [14,25]. Methacholine solutions were nebulized with a Wiesbadener Doppel inhalator, driven at an airflow of 6 L/min, generating an output of 0.1 mL per min [25]. Median mass particle size of the aerosol was 3.5 µm. Each patient used the same nebulizer for the whole study. The aerosol was inhaled during 2 min of quiet breathing with the outlet of the nebulizer in the mouth and the nose occluded with a clip. Three baseline readings were followed by inhalation of aerosolized saline. If
FEV₁ had not fallen by more than 10%, aerosolized methacholine was administered, the initial concentration being 0.031 mg/mL. Its concentration was doubled after each step. Spirometric measurements were performed 1 and 3 min after each concentration; the lowest out of these two was retained for analysis. The time interval between each step was 5 min. The procedure was terminated once FEV₁ had decreased by at least 20% or when the maximum methacholine concentration (32 mg/mL) had been reached. The PC₂₀ methacholine was calculated via linear interpolation on a logarithmic dose–response curve. If the FEV₁ had not fallen by 20% or more at the maximum methacholine concentration of 32 mg/ml, that value was substituted by 64 mg/mL.

Following methacholine challenge, the patient was allowed to recover for 2–4 h (without use of rescue medication). If FEV₁ had returned to > 90% of the pre-challenge value, an adenosine challenge was performed in the same manner as detailed above (doubling concentrations ranging from 1.563–410 mg/mL diluted in 0.9% saline) and PC₂₀ was recorded. If the FEV₁ had not fallen by 20% or more at the maximum adenosine concentration of 410 mg/ml, that value was substituted by 820 mg/mL. Patients unable to complete an adenosine challenge on the same day as the methacholine challenge returned on the following day.

**Cortisol assessments**

Twenty-four hour serum profiles were obtained from all patients after 7 days of each treatment (visits T1, T4, T7, T10 and T13 [Fig. 1]). At these visits, patients stayed at the study site for 24 hours and 5 mL of blood was drawn at 2-h intervals starting at 8.00 pm (±10 min) until 8.00 pm (±10 min) the following day. Urine was collected over 24 h at the same visits. Creatinine was also measured in the samples.
Bioanalytical methods

Blood samples for cortisol analysis were collected in tubes without anticoagulant. After collection the tubes were mixed gently and incubated for a minimum of 10 min and a maximum of 2 hours before centrifugation for 15 min at 3000 rpm at room temperature. The serum was then transferred to new tubes and stored at −20°C until analysis. Urine was collected for about 24 h, the total volume recorded and one teaspoon of sodium-azide per 2.5 L container added as a preservative. Well-mixed aliquots were stored at −20°C. Serum and urinary cortisol were measured using the GammaCoat (I^{125}) Cortisol Radioimmunoassay Kit procedure of Diasorin, which is based on the competitive binding principles of radioimmunoassay; urine was extracted before radioimmunoassay of cortisol after addition of a titrated cortisol internal standard for recovery monitoring. The limit of quantification was 0.5 µg/dL with an intra-batch coefficient of variation of 3% and an inter-batch coefficient of variation between 5.5–7.1%. For a given patient, all samples were assayed for cortisol within the same assay run. Possible interference of the trial medication with the cortisol assay antibody was assessed and no interference was found. Urinary creatinine was measured according to Jaffe (kinetic colorimetric assay) using a Roche/Hitachi MODULAR analyzer.

Assessment of bone formation makers

Blood samples to determine serum biochemical markers of bone formation were obtained on the second day of Visits T1, T4, T7, T10 and T13 at 8.00 am (±10 min) after 8 h of fasting. All samples from a given patient were assayed in a single assay run using commercial immunoassays for bone alkaline phosphatase (AP; ACCESS Immunoassay Systems, Beckman Coulter Inc, Galway, Ireland), serum osteocalcin (N-MID Osteocalcin; Osteometer Biotech A/S Copenhagen, Denmark) and serum N-terminal propeptide of type 1 procollagen (P1NP; Orion Diagnostica, Espoo, Finland).
Adverse events

Safety was assessed throughout the study by neutral questioning.

Statistical analysis

The primary variable was the 24-h serum cortisol mesor, calculated by means of the area under the curve of the 24-h serum cortisol profile (AUC$_{0-24h}$) divided by the respective time interval (8.00 pm until 8.00 pm of the following day) using the trapezoidal rule. Replacement of missing values or of outliers was not performed. A second important variable was 24-h free urine cortisol adjusted for creatinine.

To address the multiplicity issue, a strategy with a priori ordered hypotheses was applied which preserves the familywise error of the procedure at $\alpha=0.025$ (one-side). Consequently, superiority hypotheses for 24-h serum cortisol mesor and 24-h free urine cortisol adjusted for creatinine were one-sided at a significance of $\alpha=0.025$. Only if the previous null hypothesis could be rejected, the subsequent superiority test would be carried out in the following order: superiority of CIC 640 µg/day to FP 1000 µg/day for difference in serum cortisol mesor; superiority of CIC 640 µg/day to FP 1000 µg/day for change in 24-h urine cortisol adjusted for creatinine; superiority of CIC 320 µg/day to FP 500 µg/day for difference in serum cortisol mesor; superiority of CIC 320 µg/day to FP 1000 µg/day for change in 24-h urine cortisol adjusted for creatinine.

All statistical analyses were carried out with SAS (release 9.1). Serum cortisol mesor, urine cortisol variables, bone formation markers, log-transformed PC$_{20}$ and lung variables were analyzed by means of an analysis of covariance or an analysis of variance [26] with treatment, period, sequence, patient within sequence, and gender as factors. For computation of the ANOVA and ANCOVA analyses the SAS procedure PROC MIXED was utilized, using the
baseline value as continuous covariate, the patient within sequence effect as random nested factor and all other factors as fixed effects. Asthma pre-treatment and centre as factors were added for specific endpoints or analyses. T-tests of difference between the treatment least square means are given as two-sided, with an $\alpha$ level of 5%. The sample size was estimated based on findings from a previous study [14]. In the case of normally distributed difference in time-averaged cortisol levels $\text{AUC}_{0-24h}$, a sample size of 30 randomized patients was estimated to ensure a power of 80% to correctly conclude a difference in mean values of 49 nmol/L under assumption of a common standard deviation of 66.6 nmol/L. The sample size estimation was based on a two-independent-group $t$-test which provides a conservative acceptable approximation of the $t$-test for comparing least-square means utilized in the PROC MIXED procedure.

RESULTS

Patient characteristics

A total of 83 patients were screened. Of these, 51 were not eligible because of a negative methacholine or negative adenosine challenge test. The remaining 32 patients (20 females) were randomized (Table 2. Fig. 2). The first patient was included on May 27th 2003 and the last patient left the study on April 10th 2006. The characteristics of the 32 patients included in the study are summarized in Table 2. Median age was 27 years. Most patients were pretreated with a combination of a LABA and an ICS. Mean $\text{PC}_{20}$ methacholine was 2.0 mg/mL and mean $\text{PC}_{20}$ adenosine was 16.7 mg/mL. Washout period was 4 weeks on most occasions, and did not exceed 8 weeks. There were no dropouts due to asthma exacerbations. Two patients ended the study prematurely for non medical reasons and were excluded from the safety
analysis. One further patient was excluded from all analysis for erroneously using his previous ICS (FP Diskus) during the study.

**Cortisol assessments**

*Serum cortisol mesor*

Data are presented in Table 3. Both FP doses significantly suppressed cortisol secretion versus placebo, serum cortisol reaching 323.0 ± 22.6 nmol/L after FP 500 µg/day [–46.2 (95% C.I.: –83.8, –8.5) nmol/L or –10.3%] and 293.0 ± 22.3 nmol/L after FP 1000 µg/day [– 76.1 (95% C.I.: –112.9,– 39.3) nmol/L or –19.8%]. Differences in suppression between FP 1000 µg/day and both the CIC 320 µg/day [–48.0 (95% C.I.: –11.1,–84.8) nmol/L] and CIC 640 µg/day [–38.8 (95% C.I.: –1.9,–75.7) nmol/L] treatments also reached statistical significance. Neither dose of CIC had a significant suppressive effect (Table 3; Fig. 3).

*24-h urine cortisol adjusted for creatinine*

Data are presented in Table 3. Urinary cortisol excretion over 24 h adjusted for creatinine was significantly suppressed by both FP doses as compared with placebo. Neither dose of CIC demonstrated a significant effect on 24-h urinary cortisol adjusted for creatinine compared with placebo.

**Assessments of bone formation makers**

No significant differences were noted after either CIC treatment compared with placebo for any bone formation marker assessed (Table 4). However, FP 1000 µg/day caused significant decreases in P1NP (p=0.0126) and serum osteocalcin levels (p=0.0054) compared with placebo (Table 4).
**Pulmonary function measures**

FEV\textsubscript{1} remained stable over time, 90 mL being the largest difference between the highest and the lowest value. Changes from baseline in FEV\textsubscript{1} % predicted (least square means) were small for all treatments (CIC 320 µg/day, –0.2%; CIC 640 µg/day, –0.3%; FP 500 µg/day, 1.4%; FP 1000 µg/day, 3.3%; placebo, –3.1%).

**Methacholine and adenosine 5-monophosphate challenge**

Mean PC\textsubscript{20} methacholine, which was 2.0 mg/mL at inclusion increased during the study by one doubling concentration (DC), reaching 5.6 mg/mL under placebo conditions. Placebo here means that patients remained under an evening dose of CIC 160 µg throughout the study. Further improvements in airway hyperresponsiveness with the active treatments were small compared with placebo and were less than one DC (Fig. 4 – Table 5). PC\textsubscript{20} methacholine after the two FP treatments thus increased by 0.6 and 0.7 DC compared with placebo (p ≤ 0.0228), whereas the changes in hyperresponsiveness (0.3 and 0.5 DC) after CIC did not reach statistical significance (Table 5). Statistically significant differences between the CIC and FP treatments for PC\textsubscript{20} methacholine challenge were not observed.

Mean PC\textsubscript{20} adenosine, which was 16.7 mg/mL at inclusion, increased during the study by almost two DC, reaching 51.3 mg/mL under placebo conditions. The further increase in PC\textsubscript{20} adenosine with all four treatments was statistically significant compared with placebo, ranging between 1 and 2 DC (p < 0.05; Fig. 5; Table 5). Differences between the lower and the higher dose of CIC did not reach statistical significance. Likewise, the differences between the two FP doses were not statistically significant. FP 500 µg/day resulted in significantly greater improvements in PC\textsubscript{20} adenosine (one DC) compared with CIC 320 µg/day (p = 0.0238); no significant difference was seen between CIC 640 µg/day and FP 1000 µg/day, or between other doses.
Safety

In total, 22 patients experienced 56 AEs during the treatment period. The percentage of patients experiencing AEs was comparable across all treatment groups (CIC 320 µg/day, 33.3%; CIC 640 µg/day, 26.7%; FP 500 µg/day, 31.3%; FP 1000 µg/day, 22.6%; placebo, 33.3%). The majority of AEs were mild or moderate in intensity and none were assessed as definitely related to study medication. One patient in the placebo group reported two serious AEs (face edema; laryngeal edema), which were due to allergy to concomitant use of antibiotics and resolved completely.

DISCUSSION

The present study is the first placebo-controlled, crossover study assessing simultaneously the effects of ICS on cortisol secretion, bone markers and bronchial hyperresponsiveness in ICS-dependent asthma patients. The results indicate that daily doses of CIC 320 and 640 µg, given on top of a low maintenance dose of CIC 160 µg/day, did not appear to exert significant systemic effects, whereas daily doses of FP 500 and 1000 µg significantly suppressed adrenal function and bone formation markers. All active treatments improved airway responsiveness, but clinically relevant differences between the treatments were not observed.

The magnitude of the suppression of serum cortisol mesor, the primary variable, reached 10% with FP 500 µg/day and almost 20% with FP 1000 µg/day, given on top of a low maintenance dose of CIC 160 µg/day. Likewise, 24-h urinary cortisol excretion (adjusted for creatinine) was lower with FP than with placebo treatment. Substantial suppression of adrenal function after inhalation of FP has been previously reported in healthy volunteers [19] and asthmatic patients [14,20-22]. The presently observed degree of adrenal suppression with FP 1000
µg/day is somewhat smaller than the 29–34% suppression, reported previously [14,17,20], possibly because it was given on top of a low maintenance dose of inhaled CIC. The duration of the treatment cannot explain the difference between the currently and previously reported decreases in suppression, as adrenal suppression with inhaled FP is close to maximum after 7 days [27]. Possibly, the alterations in pulmonary function and airway inflammation in patients with more severe asthma resulted in a less distal lung deposition of FP, leading to a reduced pulmonary absorption, a decreased systemic bioavailability and a less pronounced adrenal suppression [22,28-30].

In contrast to FP, CIC 320 and 640 µg/day, even given on top of a low maintenance dose of CIC 160 µg/day, did not significantly alter cortisol production. Indeed, mean change in serum cortisol was –6.1% for CIC 320 µg/day and –7.9% for CIC 640 µg/day, which is in complete agreement with changes reported in previous studies [14,17]. An important finding was that differences in serum cortisol mesor between FP 1000 µg/day and the two CIC treatments reached statistical significance. Similar observations have been reported in other studies, in which the systemic effects of CIC and FP in healthy volunteers or patients with mild asthma have been assessed [14-17], be it at higher doses. It thus appears that the effects on the 24-h cortisol profile induced by FP are an intrinsic characteristic of this molecule and occur in both healthy subjects and patients with intermittent and persistent asthma. Interestingly, such effects have not been reported with CIC, even in doses as high as 1280 µg/day [14,17].

Differences in pharmacokinetic properties between FP and CIC may largely explain the more beneficial profile of CIC [31,32]. Although the clinical relevance and long-term consequences of mild adrenal suppression remain to be elucidated, the potential clinical relevance of this finding should not be underestimated. Moreover, the wide confidence intervals for serum cortisol for all comparisons indicate that the individual variability of the response of the HPA-axis and the potential occurrence of measurable systemic effects towards different doses of
different inhaled steroids cannot be neglected. Indeed, a substantial number of patients with moderate or severe asthma are treated with high doses of FP, i.e. 1000 µg/day or more, in order to reach asthma control [5,33].

Admittedly, a carry-over effect could have been missed, since this study was powered for the primary outcome. It is, however, unlikely that such a carry-over effect may have occurred, since des-ciclesonide has a half-life of just over three hours [32] and fluticasone has a half-life between 7 and 14 hours [31], whereas washout in the present study was at least 4 weeks. Moreover, cortisol secretion recovers completely 24 h after a single inhalation of 1000 µg fluticasone [24], while PC20 adenosine normalizes completely 4 weeks after discontinuation of a treatment with inhaled steroids [14,34].

Looking at markers of bone metabolism is a different way of assessing systemic effects of ICS. The current study suggests that FP 1000 µg/day significantly decreased P1NP and serum osteocalcin, whereas the lower dose of FP and both doses of ICS did not. Long-term studies with FP, in which doses from 400–750 µg/day were administered for 1–2 years demonstrated no clinically relevant effect on markers of bone formation compared with baseline [35,36]. To the best of our knowledge, no studies with higher doses of FP have been performed to date. The clinical relevance of our findings remains to be determined, although some evidence exists that long-term ICS use affects bone mineral density and increases the risk of fractures [6].

The secondary endpoints of the present study compared the clinical effects of the different treatments with those seen with placebo. Although FEV1 is often used as a marker for the clinical effect of anti-asthma drugs, this test cannot be used to establish the relative potency of ICS [14,20]. This is confirmed in the present trial, the observed differences in FEV1 between active and placebo treatments being very small. However, it has been suggested that challenges tests with methacholine [37] and adenosine [38] might be more appropriate to
differentiate the effects of high and low doses of ICS. In previous studies, CIC, inhaled via
dry powder inhaler, has demonstrated dose-dependent improvements in adenosine challenge
up to doses of 1280 µg/day [14,38], and significant protective effects versus placebo at doses
as low as 160 µg/day may be expected [12]. Likewise, dose-dependent protective effects of
FP against adenosine and methacholine challenge have been documented in previous studies
[39].

With regard to the current study, the overall effects of the ICS on bronchial
hyperresponsiveness against methacholine were small, the observed changes ranging between
0.3 and 0.7 DC. This is in keeping with previously published data [15,23,39]. In the present
study, in which FP and CIC were inhaled on top of a low maintenance dose of CIC, only the
improvement by 0.6 and 0.7 DC with the two doses of FP reached statistical significance, a
finding of little clinical relevance. These small increases did not allow us to establish the
relative potency of the four treatments. Possibly, greater and more discriminative effects
could have been obtained by prolonging each treatment to 52 weeks, a time point at which the
maximum effects of ICS on PC_{20} methacholine may be expected [40].

Adenosine-induced bronchoconstriction has been shown to be sensitive marker of airway
inflammation by promoting the release of a variety of inflammatory mediators [41], correlates
with both exhaled NO and sputum, blood and bronchial tissue eosinophilia [42] and appears
to be better suited to assess the anti-inflammatory effects of ICS than methacholine [43,44]. In
the present study, the room for improvement in PC_{20} with both CIC and FP was larger with
adenosine than with methacholine, a finding that is in line with a study in which a high dose
of ICS increased PC_{20} adenosine by 3.1 DC and PC_{20} methacholine only by 1.5 DC [43].
Nevertheless, the absolute increases in PC_{20} adenosine with FP and CIC observed in the
present study did not exceed two DC, when compared with placebo. This contrasts with
previous data by Philips [45] for FP and by Taylor and Kanniess [38,46] for CIC. CIC 400
µg/day, administered via dry powder inhaler for 14 days, thus increased PC_{20} adenosine by 2.0 DC and 2.4 DC and 1600 µg/day increased PC_{20} adenosine by 3.4 DC [38,46]. In a study with FP 1000 µg/day, PC_{20} adenosine increased by approximately 4.5 DD [45]. Differences in methodology may largely explain the observed between study differences in magnitude of effect. Firstly, the maximum treatment period of 10–12 days chosen to avoid an overall study duration in excess of 6 months, may have limited the increase in PC_{20} adenosine, which requires up to 4 weeks to reach a maximum [37,45,47]. Moreover, the administration of a maintenance dose of CIC 160 µg/day to preserve asthma control may have contributed to the unexpected, more than twofold increase in PC_{20} adenosine, compared with PC_{20} values obtained at inclusion. Possibly, the inclusion into the study improved adherence to treatment, which in daily life is known to be less than optimal in many asthma patients. This unexpected rise in DC limited the room for further improvements in PC_{20} adenosine with any of the active treatments. As the overall improvements in bronchoprotection against adenosine were small (only the difference between CIC 320 µg/day and FP 500 µg/day reached statistical significance), the relative potencies of the different treatments could not be established.

In summary, results from the current study indicate that FP 500 and 1000 µg/day exerted systemic effects in patients with moderate persistent asthma, whereas CIC 320 or 640 µg/day did not affect either biochemical markers of bone formation or serum and urinary cortisol values, if given on top of a low ciclesonide dose. Although the long-term clinical meaning of these markers remains to be investigated, they do suggest that CIC yields less systemic effects than FP in patients with moderate persistent asthma for a similar protective activity. The importance of this issue cannot be overestimated in the light of the currently accepted aims of asthma treatment [5], in which disease control with higher doses of ICS features as the primary objective.
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REFERENCES


FIGURES LEGENDS

Fig. 1. Study design
Visits T0–T1, T3–T4, T6–T7, T9–T10 and T12–T13 were separated by 7 (±0) days. Visits T0–T2,
T3–T5, T6–T8, T9–T11 and T12–T14 were separated by 9 (−2/+3) days.
CIC=ciclesonide; FP=fluticasone propionate; T=treatment visit

Fig. 2. Consort diagram showing the flow of the patients. Seq = Sequence.
83 patients were screened. 32 patients received study medication according to one of ten
sequences. Two patients discontinued the study for non medical reasons. The sequence of the
patient who did erroneously continue to use his Diskus FP on top of his study medication, was
a posteriori called sequence 11, since that treatment did not correspond with one of ten
sequences originally scheduled before the start of the study.

Fig. 3. Mean serum cortisol mesor (nmol/L) following placebo (PLA), ciclesonide 320
µg/day (CIC 320), ciclesonide 640 µg/day (CIC 640), fluticasone propionate 500 µg/day
(FP 500) or fluticasone propionate 1000 µg/day (FP 1000). All treatment groups were
administered CIC 160 µg once daily in the evening plus salmeterol 50 µg twice daily. Data
are presented as at least squares (LS) mean ± standard error of the LS mean. *p<0.01 versus
placebo; †p≤0.0057 versus CIC 320 µg/day; ‡p=0.0197 versus CIC 640 µg/day.

Fig. 4. PC_{20} methacholine at intake and after placebo, ciclesonide and fluticasone. All
treatment groups were administered CIC 160 µg once daily in the evening plus salmeterol 50
µg twice daily. Same abbreviations as in Fig. 3.
Fig. 5. **PC\textsubscript{20} adenosine at intake and after placebo, ciclesonide and fluticasone.** All treatment groups were administered CIC 160 µg once daily in the evening plus salmeterol 50 µg twice daily. Same abbreviations as in Fig. 3.
Table 1. List of the 10 permutations of order, used for the 5 treatments.

<table>
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<th>Sequence</th>
<th>TI</th>
<th>TII</th>
<th>TIII</th>
<th>TIV</th>
<th>TV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CIC 320</td>
<td>CIC 640</td>
<td>PLAC</td>
<td>FP 500</td>
<td>FP 1000</td>
</tr>
<tr>
<td>2</td>
<td>CIC 320</td>
<td>PLAC</td>
<td>CIC 640</td>
<td>FP 1000</td>
<td>FP 500</td>
</tr>
<tr>
<td>3</td>
<td>CIC 640</td>
<td>CIC 320</td>
<td>FP 500</td>
<td>PLAC</td>
<td>FP 1000</td>
</tr>
<tr>
<td>4</td>
<td>CIC 640</td>
<td>FP 500</td>
<td>CIC 320</td>
<td>FP 1000</td>
<td>PLAC</td>
</tr>
<tr>
<td>5</td>
<td>FP 500</td>
<td>CIC 640</td>
<td>FP 1000</td>
<td>CIC 320</td>
<td>PLAC</td>
</tr>
<tr>
<td>6</td>
<td>FP 500</td>
<td>FP 1000</td>
<td>CIC 640</td>
<td>PLAC</td>
<td>CIC 320</td>
</tr>
<tr>
<td>7</td>
<td>FP 1000</td>
<td>FP 500</td>
<td>PLAC</td>
<td>CIC 640</td>
<td>CIC 320</td>
</tr>
<tr>
<td>8</td>
<td>FP 1000</td>
<td>PLAC</td>
<td>FP 500</td>
<td>CIC 320</td>
<td>CIC 640</td>
</tr>
<tr>
<td>9</td>
<td>PLAC</td>
<td>CIC 320</td>
<td>FP 1000</td>
<td>CIC 640</td>
<td>FP 500</td>
</tr>
<tr>
<td>10</td>
<td>PLAC</td>
<td>FP 1000</td>
<td>CIC 320</td>
<td>FP 500</td>
<td>CIC 640</td>
</tr>
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</table>
Table 2. Baseline demographics and characteristics.

<table>
<thead>
<tr>
<th></th>
<th>N=32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years</td>
<td>27</td>
</tr>
<tr>
<td>Range</td>
<td>18–59</td>
</tr>
<tr>
<td>Mean (± SD) weight, kg</td>
<td>70 ± 16</td>
</tr>
<tr>
<td>Mean (± SD) height, cm</td>
<td>171 ± 9</td>
</tr>
<tr>
<td>Gender, n</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
</tr>
<tr>
<td>Race, n</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>31</td>
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<tr>
<td>Black</td>
<td>1</td>
</tr>
<tr>
<td>ICS pre-treatment, n (%)</td>
<td></td>
</tr>
<tr>
<td>ICS</td>
<td>12</td>
</tr>
<tr>
<td>ICS/LABA</td>
<td>20</td>
</tr>
<tr>
<td>Smoking Status, n (%)</td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>19</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>11</td>
</tr>
<tr>
<td>Current smokers</td>
<td>2</td>
</tr>
<tr>
<td>Mean (± SD) FEV\textsubscript{1} % predicted*</td>
<td>84.9 ± 13.2</td>
</tr>
<tr>
<td>Mean PC\textsubscript{20} AMP (mg/ml)</td>
<td>16.7 (2.0–60)</td>
</tr>
<tr>
<td>Mean PC\textsubscript{20} MCh (mg/ml)</td>
<td>2.0 (0.1–8.0)</td>
</tr>
</tbody>
</table>

*Taken at randomization

SD=standard deviation; ICS=inhaled corticosteroid; LABA=long-acting β\textsubscript{2}-agonist; FEV\textsubscript{1}=forced expiratory volume in 1 second; PC\textsubscript{20}=provocative concentration leading to a 20% decrease in FEV\textsubscript{1}; AMP=adenosine 5-monophosphate; MCh=methacholine.
Table 3. Effects of treatments on serum cortisol mesor and urine cortisol, adjusted for creatinine. Placebo = maintenance dose of CIC 160 µg/day).

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>CIC 320 µg/day</th>
<th>CIC 640 µg/day</th>
<th>FP 500 µg/day</th>
<th>FP 1000 µg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cortisol mesor, nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=27</td>
<td>N=27</td>
<td>N=27</td>
<td>N=27</td>
<td>N=27</td>
<td>N=29</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>381.2 ± 98.5</td>
<td>352.8 ± 110.4</td>
<td>341.6 ± 95.9</td>
<td>332.9 ± 94.2</td>
<td>304.4 ± 150.6</td>
</tr>
<tr>
<td>LSmean ± SEM</td>
<td>369.2 ± 22.6</td>
<td>341.0 ± 22.7</td>
<td>331.8 ± 22.7</td>
<td>323.0 ± 22.6</td>
<td>293.0 ± 22.3</td>
</tr>
<tr>
<td>Difference vs. placebo</td>
<td>-</td>
<td>-28.2 ± 18.8</td>
<td>-37.3 ± 18.8</td>
<td>-46.2 ± 19.0</td>
<td>-76.1 ± 18.6</td>
</tr>
<tr>
<td>95% CI</td>
<td>-</td>
<td>-65.5, 9.2</td>
<td>-74.7, 0.0</td>
<td>-83.8, -8.5</td>
<td>-112.9, -39.3</td>
</tr>
<tr>
<td>p-value</td>
<td>-</td>
<td>0.0251</td>
<td>0.0687</td>
<td>0.0084</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Difference vs. FP 1000 µg/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSmean ± SEM</td>
<td>76.1 ± 18.6</td>
<td>48.0 ± 18.6</td>
<td>38.8 ± 18.6</td>
<td>30.0 ± 18.7</td>
<td>-</td>
</tr>
<tr>
<td>95% CI</td>
<td>39.3, 112.9</td>
<td>11.1, 84.8</td>
<td>1.9, 75.7</td>
<td>-7.2, 67.1</td>
<td>-</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0001</td>
<td>0.0057</td>
<td>0.0197</td>
<td>0.0563</td>
<td>-</td>
</tr>
<tr>
<td>Change in serum cortisol to placebo, %</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=26</td>
<td>N=26</td>
<td>N=25</td>
<td>N=27</td>
<td></td>
<td>N=27</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>-6.1 ± 26.1</td>
<td>-7.9 ± 18.5</td>
<td>-10.3 ± 20.9</td>
<td>-19.8 ± 28.0</td>
<td></td>
</tr>
<tr>
<td>24-hour urine cortisol adjusted for creatine, nmol/mmol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=25</td>
<td>N=26</td>
<td>N=27</td>
<td>N=27</td>
<td>N=28</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>25.74 ± 17.24</td>
<td>22.95 ± 10.17</td>
<td>23.72 ± 10.75</td>
<td>20.49 ± 7.49</td>
<td>20.74 ± 10.93</td>
</tr>
<tr>
<td>LSmean ± SEM after treatment</td>
<td>25.04 ± 2.44</td>
<td>22.12 ± 2.43</td>
<td>23.32 ± 2.39</td>
<td>20.06 ± 2.39</td>
<td>19.80 ± 2.37</td>
</tr>
<tr>
<td>Difference vs. placebo,</td>
<td>-</td>
<td>-2.92 ± 2.38</td>
<td>-1.72 ± 2.35</td>
<td>-4.98 ± 2.36</td>
<td>-5.24 ± 2.32</td>
</tr>
<tr>
<td>LSmean ± SEM</td>
<td>-</td>
<td>-7.64, 1.80</td>
<td>-6.38, 2.94</td>
<td>-9.66, -0.30</td>
<td>-9.84, -0.64</td>
</tr>
<tr>
<td>p-value</td>
<td>-</td>
<td>0.1111</td>
<td>0.2326</td>
<td>0.0186</td>
<td>0.0130</td>
</tr>
</tbody>
</table>

For all safety parameters: the data provided are for the restricted safety analysis, excluding one patient.
CIC=ciclesonide; FP=fluticasone propionate; LS=least squares; SEM=standard error of the LSmean; CI=confidence interval; SD=standard deviation.
Table 4. Least squares mean changes in bone formation markers compared with placebo (= maintenance dose of CIC 160 µg/day).

<table>
<thead>
<tr>
<th></th>
<th>CIC 320 µg/day (N=27)</th>
<th>CIC 640 µg/day (N=27)</th>
<th>FP 500 µg/day (N=27)</th>
<th>FP 1000 µg/day (N=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P1NP (µg/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS mean ± SEM</td>
<td>−2.7 ± 4.1</td>
<td>0.8 ± 4.1</td>
<td>−3.3 ± 4.2</td>
<td>−10.4 ± 4.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>−10.9, 5.5</td>
<td>−7.4, 9.0</td>
<td>−11.6, 4.9</td>
<td>−18.4, −2.3</td>
</tr>
<tr>
<td>p-value</td>
<td>0.5156</td>
<td>0.8376</td>
<td>0.4258</td>
<td>0.0126</td>
</tr>
<tr>
<td><strong>Serum osteocalcin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/mL)</td>
<td>0.7 ± 1.2</td>
<td>0.0 ± 1.2</td>
<td>−1.8 ± 1.2</td>
<td>−3.3 ± 1.2</td>
</tr>
<tr>
<td>LS mean ± SEM</td>
<td>−1.7, 3.0</td>
<td>−2.4, 2.3</td>
<td>−4.2, 0.6</td>
<td>−5.6, −1.0</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.5814</td>
<td>0.9799</td>
<td>0.1312</td>
<td>0.0054</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bone specific AP</strong></td>
<td></td>
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</tr>
<tr>
<td>(µg/L)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LS mean ± SEM</td>
<td>0.4 ± 0.4</td>
<td>−0.1 ± 0.4</td>
<td>0.3 ± 0.4</td>
<td>0.0 ± 0.4</td>
</tr>
<tr>
<td>95% CI</td>
<td>−0.4, 1.2</td>
<td>−0.9, 0.7</td>
<td>−0.5, 1.1</td>
<td>−0.7, 0.8</td>
</tr>
<tr>
<td>p-value</td>
<td>0.3304</td>
<td>0.8710</td>
<td>0.4473</td>
<td>0.9067</td>
</tr>
</tbody>
</table>

CIC=ciclesonide; FP=fluticasone propionate; P1NP=N-terminal propetide of type 1 procollagen; AP=alkaline phosphatase. P-values are set versus placebo.
Table 5. Change in PC$_{20}$ methacholine and PC$_{20}$ adenosine (doubling doses) compared with placebo (= maintenance dose of CIC 160 µg/day).

<table>
<thead>
<tr>
<th>Change in PC$_{20}$ (doubling concentrations) versus placebo</th>
<th>CIC 320 µg/day (N=29)</th>
<th>CIC 640 µg/day (N=29)</th>
<th>FP 500 µg/day (N=30)</th>
<th>FP 1000 µg/day (N=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methacholine LS mean ± SEM</td>
<td>0.3 ± 0.3</td>
<td>0.5 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.3, 0.8</td>
<td>0.0, 1.1</td>
<td>0.1, 1.2</td>
<td>0.1, 1.3</td>
</tr>
<tr>
<td>p-value</td>
<td>0.3356</td>
<td>0.0645</td>
<td>0.0228</td>
<td>0.0145</td>
</tr>
<tr>
<td>Adenosine LS mean ± SEM</td>
<td>0.9 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.1, 1.7</td>
<td>0.6, 2.4</td>
<td>1.0, 2.6</td>
<td>0.6, 2.2</td>
</tr>
<tr>
<td>p-value</td>
<td>0.0218</td>
<td>≤0.0001</td>
<td>≤0.0001</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

PC$_{20}$=provocative concentration leading to a 20% decrease in FEV$_1$; LS=least squares; SEM=standard error; CIC=ciclesonide; FP=fluticasone propionate.
Fig. 1

<table>
<thead>
<tr>
<th>Run-in period (4–6 weeks)</th>
<th>Treatment period I (9 days)</th>
<th>Washout period I (4–12 weeks)</th>
<th>Treatment period II (9 days)</th>
<th>Washout period II (4–12 weeks)</th>
<th>Treatment and washout periods III–V (9 days)</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₀</td>
<td>T₀</td>
<td>T₁, T₂</td>
<td>T₃</td>
<td>T₄, T₅</td>
<td>T₆–T₆/T₆₉</td>
<td></td>
</tr>
</tbody>
</table>

- CIC 320 µg/day
- CIC 640 µg/day
- FP 500 µg/day
- FP 1000 µg/day
- Placebo
- CIC 320 µg/day
- CIC 640 µg/day
Fig. 2

Screened n=83

Enrolled n=32

Randomized n=32

Not eligible for inclusion n=51

Withdrawn prior to randomization n=0

Patient allocated to one of the respective treatment sequences and took at least one dose of study medication

Seq 1 n=3  Seq 2 n=2  Seq 3 n=3  Seq 4 n=4  Seq 5 n=3  Seq 6 n=3  Seq 7 n=3  Seq 8 n=4  Seq 9 n=3  Seq 10 n=3  Seq 11* n=1

Completed n=3  Completed n=2  Completed n=3  Completed n=4  Completed n=2  Completed n=3  Completed n=2  Completed n=4  Completed n=3  Completed n=1

Discontinued study n=1

Discontinued study n=1
Fig. 3

![Bar chart showing serum cortisol levels for different groups](image)

- PBO
- CIC320
- CIC640
- FP500
- FP1000

Serum cortisol mesor (nmol/L)

*Statistical significance indicated by symbols.*
Fig. 4

PC_{50}, methacholine (mg/mL)

<table>
<thead>
<tr>
<th></th>
<th>Baseline (Base)</th>
<th>PBO</th>
<th>CIC320</th>
<th>CIC640</th>
<th>FP500</th>
<th>FP1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>2.0</td>
<td>5.6</td>
<td>6.9</td>
<td>8.2</td>
<td>8.4</td>
<td>9.3</td>
</tr>
</tbody>
</table>
Fig. 5

PC_{20} adenosine (mg/mL)

- Base: 16.7
- PBO: 51.3
- CIC320: 103.3
- CIC640: 164.6
- FP500: 179.6
- FP1000: 139.7