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Original article

Anti-inflammatory effects of once daily low dose inhaled ciclesonide in mild to moderate asthmatic patients

Background: Ciclesonide exhibits clinical efficacy at 160 μg (ex-actuator) once daily but the anti-inflammatory effects at this dose are not known. We wished to know whether 4 weeks therapy with ciclesonide pMDI 160 μg once daily in the morning exhibited significant anti-inflammatory effects.
Methods: Seventeen patients with mild persistent asthma (FEV_1 3.35 l) were recruited into a double-blind placebo-controlled randomized crossover study. Measurements were made after ciclesonide and placebo treatment as well as after run-in and washout periods, for adenosine monophosphate (AMP) bronchial challenge (primary variable), exhaled nitric oxide (NO) and induced sputum (in a subgroup).
Results: The mean (SEM) AMP bronchial challenge PC_{20} following ciclesonide (140 (63) mg/ml) was significantly ($P < 0.001$) increased compared with placebo (17 (8) mg/ml), run-in (13 (5) mg/ml) and washout (9 (3) mg/ml) periods. This amounted to an eightfold (CI: 5.3–12.0) for ciclesonide *vs* placebo. Likewise, there were significant improvements in exhaled NO levels and a significant reduction in induced sputum eosinophil cell counts.
Conclusion: We have shown that inhaled ciclesonide given at 160 μg once daily in the morning exhibits significant anti-inflammatory effects that are in keeping with the previously described clinical effects.

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Key words: adenosine monophosphate; asthma; ciclesonide; exhaled nitric oxide; induced sputum.

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Inhaled corticosteroids are the first-line anti-inflammatory therapy in the management of asthma (1). They work by altering the production of genes involved in the inflammatory process, thereby reducing the synthesis of inflammatory proteins and cytokines (2). Corticosteroids have been shown to reduce the numbers of inflammatory cells and their inflammatory action, basement membrane thickness and airway hyperresponsiveness.

Ciclesonide is a new corticosteroid for use in asthma. It is converted by esterases within the lung to its active metabolite, desisobutyryl-ciclesonide which has 100 times the affinity of the parent compound at the glucocorticoid receptor (3). As such it is a pro-drug and therefore has the advantage of minimizing the local adverse effects (4). Ciclesonide is formulated with HFA-134a as a propellant in a metred dose inhaler which has been shown to have a high pulmonary respirable fraction and low oropharyngeal deposition.

Bronchial hyperresponsiveness is a cardinal feature of asthma and can be measured quantitatively by bronchial challenge testing. This can be performed with a direct stimulus such as methacholine, or an indirect stimulus such as adenosine monophosphate (AMP). AMP, which acts via priming of airway mast cells to release inflammatory mediators (5), has been shown to be more sensitive than other challenges (6) and is more closely related to airway inflammation and atopic status (7).

Ciclesonide's anti-inflammatory properties have been evaluated, in terms of induced sputum eosinophil count and AMP bronchial challenge, in a parallel group dose-ranging study by Taylor et al. (8). In that study 30 patients, who were steroid naïve, were randomized to receive ciclesonide at daily (ex-actuator) doses of 80, 320 and 1280 μg/day. There were significant effects only at the two highest doses against AMP bronchial challenge. However, a dose of 160 μg once daily was not evaluated and ciclesonide was given in divided doses in the morning and evening. Postma et al. (9) and more recently Chapman et al. (10) have demonstrated efficacy in terms of spirometry and symptoms with ciclesonide 160 μg once daily, but the anti-inflammatory efficacy of this dose has not been evaluated using AMP bronchial challenge.

We therefore wished to evaluate the anti-inflammatory and anti-asthmatic efficacy of low dose inhaled ciclesonide (160 μg once daily) in comparison with placebo in

Abbreviations: AMP, adenosine monophosphate; ANOVA, analysis of variance; CI, confidence interval; FEV_1, forced expiratory volume in 1 s; IQR, inter-quartile range; NO, nitric oxide; PC_{20}, provocation concentration causing 20% fall in FEV_1; SEM, standard error of mean.

steroid naïve subjects in terms of change in AMP bronchial hyperresponsiveness. This study is particularly relevant, as ciclesonide has recently been licensed for use in Europe in mild to moderate asthmatic subjects at a dose of 160 μg once daily.

Methods

Patients

Twenty subjects with a history of mild to moderate persistent asthma were enrolled from local advertisement. One subject withdrew for personal reasons, one subject withdrew due to a respiratory tract infection during the run-in period and a third withdrew after failing to return to baseline (AMP PC_{20}) following the 2 weeks washout. The remaining 17 subjects completed the study (Table 1). All subjects were aged between 18 and 71 years, atopic, nonsmokers, and controlled for at least 4 weeks and demonstrated bronchial hyperresponsiveness to methacholine and AMP (Table 1). None had any cardiac or pulmonary disease other than asthma and none were receiving medication for upper or lower airway disease, other than inhaled short acting bronchodilators. Induced sputum eosinophil counts were analysed in a subgroup of seven patients. Apart from FEV_1, there was no significant difference in any of the demographic values (including bronchial hyperresponsiveness to AMP and methacholine) when comparing subjects who were or were not in the induced sputum subgroup (Table 1). The FEV_1 was significantly higher in subjects who were in the induced sputum subgroup (3.84 l *vs* 3.00 l).

Design

This was a randomized, double-blind, two-way crossover study, with a 1-week run-in and a 2-week washout phase. Randomization was performed by a pharmacist and the code sealed in an envelope. Patients were assessed on five different occasions (at screening and after treatment periods, run-in and washout) throughout the study. Each occasion comprised of two visits which were 48 h apart. Visits were between 08:00 and 12:00 hours, 2 h within respective baseline measurements for each period and 4 h after taking medication. On day 1 of the screening visit, skin prick testing, spirometry and AMP bronchial challenge were measured and on the second day, methacholine challenge and sputum induction (in subgroup), were performed. On subsequent assessments, exhaled nitric oxide (NO) and sputum induction were taken on the first day. Exhaled NO was performed before sputum induction. Clinical assessment for adverse events, domiciliary recordings and AMP bronchial challenge were performed on the second day. A urinary pregnancy test was performed at entry to and exit from the study.

Randomized treatment was with 4 weeks of ciclesonide (Aventis Pharmaceuticals Inc., Bridgewater, NJ, USA) 160 μg (ex actuator) once daily at 08:00 hours, as two puffs of HFA-134a metred dose inhaler 80 μg per actuation, or inhaled placebo metred dose inhaler two puffs once daily. Patients were instructed on how to use their inhaler device prior to enrolment and their inhaler technique was checked at each visit. Adherence was assessed by asking patients to fill out a chart when medication was taken. Patients were given inhaled salbutamol to be used as rescue medication for the duration of the study. Ethical approval for the study was granted by McMaster University Research Ethics Board and all patients provided written informed consent.

Table 1. Demographic details

Induced sputum	Age (years)	Gender	Asthma duration (years)	Skin prick +ve	FEV_1 (l)	PC_{20} MCh (mg/ml)	PC_{20} AMP (mg/ml)
Y	19	Male	2	3	3.44	0.3	18.8
Y	24	Male	9	5	3.76	0.1	28.0
Y	20	Male	16	8	3.67	0.7	7.0
Y	27	Female	15	6	2.85	0.3	62.2
Y	20	Female	12	4	3.95	0.4	38.8
Y	25	Male	20	6	4.11	0.3	11.5
Y	22	Male	12	4	5.08	1.6	27.3
N	29	Male	3	3	3.71	1.1	14.6
N	71	Male	1	7	3.16	1.5	7.5
N	52	Female	30	2	1.83	0.4	51.6
N	47	Female	35	5	3.15	0.1	18.9
N	42	Female	18	1	2.93	2.5	5.7
N	23	Female	5	7	2.54	0.1	0.2
N	22	Female	17	2	2.83	0.2	10.3
N	22	Male	14	6	4.07	0.8	11.1
N	21	Female	11	6	2.52	0.3	46.9
N	18	Female	10	2	3.31	0.6	2.9
Sputum	22.4 (4.3)	5 Male	12.3 (2.7)	5 (3)	3.84 (0.15)	0.4 (0.1)	22.4 (8.9)
Nonsputum	34.7 (4.3)	3 Male	14.4 (2.7)	4 (4)	3.00 (0.15)	0.4 (0.1)	8.4 (3.4)
Total	29.6 (3.6)	8 Male	13.5 (2.2)	5 (3)	3.35 (0.18)	0.4 (0.1)	12.6 (4.3)
P-value	0.090	0.092	0.653	0.246	0.021	0.745	0.156

Mean (standard error of mean) age, asthma duration and forced expiratory volume in 1 s (FEV_1), median (interquartile range) for number of skin prick positives and geometric mean (standard error of mean) of methacholine (MCh) and adenosine monophosphate (AMP) PC_{20} in all patients and the subgroup with induced sputum analysis. The *P*-value for comparison of subjects providing and not-providing induced sputum.

Measurements

Spirometry was performed according to European Respiratory Society Guidelines (11) using a Microlab spirometer (Micro Medical Ltd, Rochester, Kent, UK).

Methacholine challenge testing and AMP bronchial challenge were performed as previously described (12, 13), after patients had withheld their short acting reliever medication for 8 h. Methacholine (0.125–8 mg/ml) and AMP (0.09–800 mg/ml) were administered in doubling cumulative doses at 5-min intervals until a fall in forced expiratory volume in 1 s (FEV_1) greater than or equal to 20% occurred. The provocation concentration causing 20% fall in FEV_1 (PC_{20}) was calculated by interpolation of the steep part of the log dose–response curve and expressed as noncumulative units. If the FEV_1 did not fall by 20% after the maximum dose was administered a value of 16 and 1600 mg/ml was assigned for methacholine and adenosine monophosphate, respectively.

Exhaled nitric oxide was measured using a NIOX nitric oxide analyzer (Aerocrine, Chicago, IL, USA), with an expiratory flow rate of 0.05 l/s (14). The mean of three separate measures of nitric oxide was used in the analysis. The analyser was calibrated daily using a cylinder of nitric oxide at a concentration of 200 ppb.

Sputum induction, preceded by spirometry before and 10 min after 200 μg of inhaled salbutamol, was performed by nebulizing increasing concentrations (3%, 4% and 5%) of hypertonic saline each for 7 min. Specimens were processed within 2 h as described by Pizzichini et al. (15). Total cell count was calculated in a Neubauer haemocytometer and cell viability was determined by the tryptan blue exclusion method. Cytospins were prepared using a Shandon III cytocentrifuge (Shandon Southern Instruments, Sewickley, PA, USA) and stained by Wright's stain for differential cell count. Four hundred nonsquamous cells were counted and the results were expressed as a percentage of the total.

Measurement of spirometry (peak expiratory flow and FEV_1) using a Koko Peak Pro (Ferraris Cardiorespiratory, Louisville, CO, USA) was made at 08:00 and 22:00 hours at home and the data downloaded to a desktop computer. Patients also recorded their asthma symptoms (breathlessness, wheeze, chest tightness, cough, sputum) according to a eight-point scale (zero indicating maximal symptoms and seven indicating no symptoms), the number of night-time or early morning awakenings due to asthma and their requirement for rescue inhaler requirement with β_2 agonists on a daily basis.

Statistical analysis

The sample size required to detect a 1 doubling dose difference (standard deviation = 1.34 doubling doses) in AMP PC_{20} (the primary endpoint) between placebo and ciclesonide (16) with a power of 80% and alpha error of 0.05 (two-tailed) was 17 patients. Comparisons for demographic values between those subjects providing and not-providing sputum samples were performed by Student's *t*-test (age, asthma duration, FEV_1, methacholine PC_{20}, AMP PC_{20}), Wilcoxon signed ranks test (skin prick positives), and chi-squared test (Gender). In two patients, all data were downloaded incorrectly from the Koko spirometers. In a further two patients there were missing data for ciclesonide treatment ($n = 1$) and placebo ($n = 1$) and therefore the analysis was performed using the data from 13 subjects. As there were data missing from the run-in period, the average of the data from the run-in and washout periods was used in the analysis.

Domicillary data (symptom scores, β_2 agonist requirements, peak flow, spirometry) were averaged for the last 5 days of each treatment period for the purposes of analysis. Normally distributed data were analysed by parametric methods and are expressed as mean with standard error of mean (SEM). Otherwise, nonparametric methods were used and these data are expressed as median with interquartile range (IQR).

Univariate analysis of variance (ANOVA) was used to compare the treatment effect on AMP PC_{20}, exhaled nitric oxide, laboratory FEV_1, domiciliary peak flow and FEV_1 and symptom scores with treatment as the fixed factor and subject and period as random factors. This was followed by Bonferroni multiple range testing set at 95% confidence limits. Sputum eosinophil and neutrophil percentage data were analysed by the Friedman's test, followed by Wilcoxon signed ranks test.

Results

Laboratory data

There were no significant differences between the run-in period, washout period or placebo for any of the endpoints examined (Fig. 1). For AMP PC_{20}, there was a significant difference between ciclesonide and placebo (Figs 1 and 2), which amounted to an 8.0 (CI: 5.3–12.0) fold difference or a 3.0 (1.7–4.3) doubling concentration difference. The difference between ciclesonide and placebo was also significant for exhaled nitric oxide [47 (95% CI: 15–81) ppb] (Fig. 1). Likewise, for sputum eosinophil count, there was a significant difference between ciclesonide (4.5 (6.4)%) and placebo (6.9 (16.8)%) ($P = 0.028$), run-in (6.0 (21.0)%) ($P = 0.018$) and washout (6.5 (15.7)%) ($P = 0.043$) periods (Fig. 1). There was no significant difference for percentage sputum neutrophil counts between ciclesonide (43.7 (28.5)%) and placebo (33.1 (27.3)%) ($P = 0.61$), run-in (37.4 (34)%) ($P = 1.0$) and washout (32.4 (32.5)%) ($P = 0.87$) periods. There was no difference between treatments in terms of laboratory FEV_1 [0.20 (95% CI: −0.30–0.42) l] (Fig. 1).

Domiciliary data

There was no significant difference between the two baseline phases and treatment periods in terms of domiciliary peak expiratory flow [run-in/washout 423 (32) l/min, ciclesonide 431 (35) l/min, placebo 430 (30) l/min] or domiciliary FEV_1 [run-in/washout 3.04 (0.35) l, ciclesonide 3.12 (0.37) l, placebo 2.96 (0.32) l]. Likewise, there was no significant difference between any of the assessment periods for asthma symptoms (Table 2) although there was a trend to improvement with ciclesonide.

Adverse events

There were no serious adverse events. Of the 20 patients enrolled into the study, five had no adverse events, and the remaining 15 subjects had a total of 18 adverse events. Increased breathlessness or an exacerbation of asthma occurred in two patients during the run-in, washout and placebo period and one patient during ciclesonide therapy. Nasal congestion occurred once during the washout and once during the placebo period. During ciclesonide

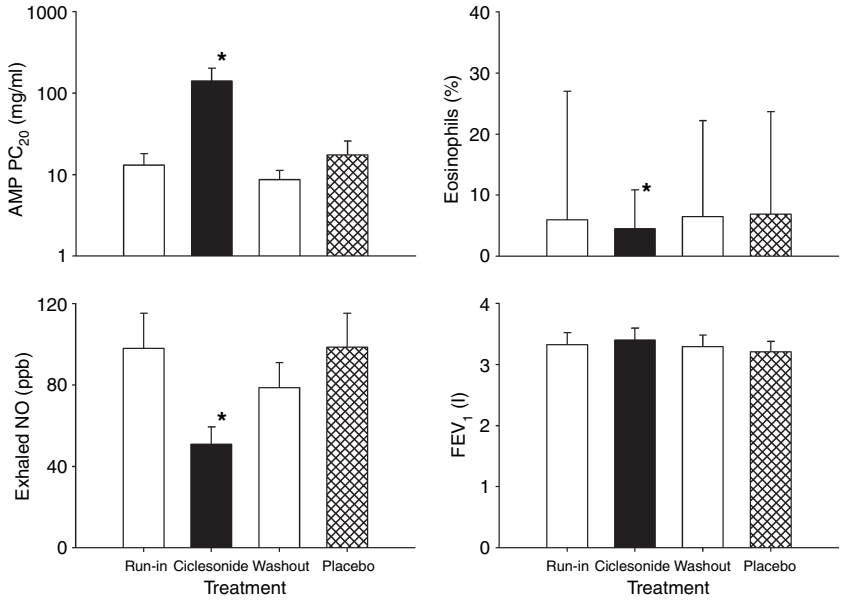

Figure 1. Geometric mean with standard error of mean (SEM) for adenosine monophosphate (AMP) provocation concentration causing 20% fall in (PC_{20}), median with interquartile range induced sputum percentage (%) eosinophilia, and means with SEM for exhaled nitric oxide (NO) and forced expiratory volume in 1 s (FEV_1). *Significant difference between ciclesonide and all other assessment periods.

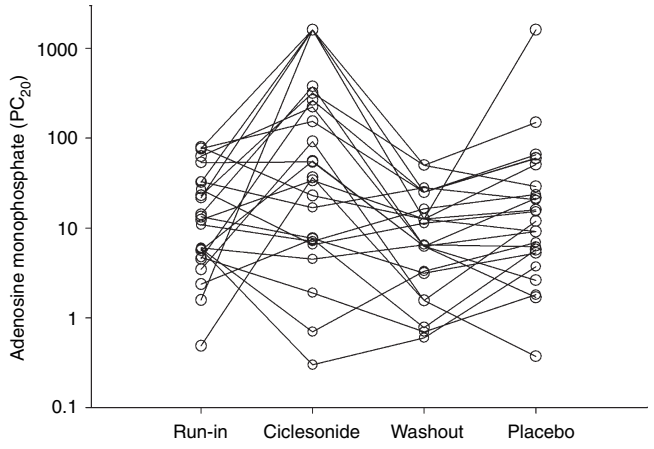

Figure 2. Scatter plot for AMP PC_{20}. Solid line joins the values of an individual patient.

Table 2. Diary card symptom scores

	Run-in	Ciclesonide	Washout	Placebo
Breathlessness	6.7 (0.1)	6.8 (0.1)	6.6 (0.2)	6.6 (0.1)
Wheeze	6.5 (0.2)	6.6 (0.2)	6.5 (0.2)	6.5 (0.1)
Chest tightness	6.5 (0.1)	6.8 (0.1)	6.6 (0.2)	6.6 (0.1)
Cough	6.5 (0.2)	6.8 (0.1)	6.5 (0.2)	6.5 (0.2)
Sputum	6.8 (0.1)	6.7 (0.2)	6.6 (0.1)	6.6 (0.2)
Night-time/early morning wakening (number)	0.1 (0.1)	0.0 (0.0)	0.3 (0.2)	0.2 (0.1)
β_2 rescue medication (puffs)	0.5 (0.1)	0.2 (0.1)	0.5 (0.2)	0.7 (0.3)

Mean (SEM) for symptom scores, number of early morning wakenings and puffs of reliever medication. Symptom scores range from 0 to 7 (7 = no symptoms, 0 = maximal symptoms), wakenings are number per day and reliever requirement are puffs per day. There was no significant difference between assessment periods for any measurement.

therapy there was one incident each of tooth abscess, headache, diarrhoea, sore throat which lasted for 1 day, laryngitis and upper respiratory tract infection. During the washout one person had oral lesions which resolved with mouth washing, and during the placebo period one person complained of neck flushing and cough. None of the adverse events occurring during ciclesonide therapy were thought to be related to this treatment.

Discussion

We have shown that inhaled ciclesonide given at the low dose of 160 μg (ex-valve) once daily produced significant improvements in measures of airway inflammation namely adenosine monophosphate bronchial challenge, exhaled nitric oxide and induced sputum eosinophil count. For AMP this amounted to an eightfold difference between ciclesonide and placebo treatment. These data are complimentary to the previously demonstrated clinical effects with once daily dosing of low dose ciclesonide (9, 10).

The eightfold difference between ciclesonide and placebo for AMP PC_{20} equates to a 3 doubling concentration shift in response. This finding is comparable with the results of other studies which compared the efficacy of inhaled corticosteroids using AMP bronchial challenge. When taken in two divided doses, daily (ex-actuator) doses of 80, 360 and 1280 μg ciclesonide

reduced airway hyperresponsiveness to AMP by 1.6, 2.0 and 3.4 doubling doses, respectively (8). Budesonide given at daily (ex-valve) doses of 100, 400 and 1600 μg, also in divided doses, reduced hyperresponsivess by 2, 2.75 and 4 doubling doses (17). Fluticasone propionate has been shown to reduce hyperresponsiveness by 1.7 doubling concentrations at 500 μg twice daily (18) and 4.5 doubling doses at 750 μg twice daily (19). Ciclesonide 160 μg once daily therefore exhibited similar bronchoprotection to other inhaled corticosteroids, evaluated in different studies, when given twice daily at higher doses.

Exhaled nitric oxide is particularly sensitivity to the effects of inhaled corticosteroids (6) with many studies showing significant suppression at low or moderate doses. Other authors have examined the effect of ciclesonide therapy on exhaled nitric oxide concentration. Lee et al. showed significant suppression with high dose ciclesonide (1280 μg/day) (20), but not at moderate dose (320 μg/day) (21). Whereas Kanniess et al. (22) showed significant suppression with 320 μg/day. This would be in keeping with our finding of significant suppression at a dose of 160 μg/day.

There is a great deal of interest in prescribing inhaled corticosteroids on a once daily basis. This is intended to increase adherence to therapy without any loss of clinical efficacy. Postma et al. (9) have evaluated the effect of once daily 160 μg ciclesonide given in either the morning or evening for 8 weeks. They found morning administration improved laboratory spirometry, daily symptoms and rescue medication, but not daily peak flow whereas all of the measures improved with evening administration. Chapman et al. (10) have recently evaluated both 160 and 640 μg once daily in patients with persistent asthma also in terms of peak expiratory flow and spirometry and showed significant improvements with both doses compared with placebo. Kanniess et al. (22) have compared inhaled ciclesonide and budesonide 400 μg once daily in the morning for 2 weeks. Both budesonide and ciclesonide had significant effects on exhaled nitric oxide and AMP PC_{20} (with doubling concentration shifts of 2.8 and 2.4, respectively) but only ciclesonide significantly reduced sputum eosinophil levels. In the study by Aziz et al. (23), once daily dosing of budesonide given at doses of 200 and 800 μg/day resulted in doubling concentration shifts of 2.4 and 3.3, respectively, for changes in AMP *vs* placebo. More recently, Lee et al. (21) compared ciclesonide 320 μg (ex-actuator) once daily in the morning and fluticasone propionate 220 μg (ex-actuator) twice daily using methacholine bronchial challenge testing and showed no significant difference between these corticosteroids.

Although significant differences were detected with all measures of airway inflammation, when compared with placebo, inflammation was not eradicated, with mean levels of AMP PC_{20}, sputum exhaled NO and sputum eosinophil count all being higher than normal limits for healthy subjects. Furthermore, we did not demonstrate any significant improvement in laboratory spirometry, although this would be expected given our sample size and the fact that the subjects all had mild asthma. Likewise, we found no significant improvement in terms of domiciliary data; although, there was a trend to improvement with ciclesonide with all measures. Domiciliary spirometry was recorded twice daily at home using a hand-held electronic portable spirometer (Koko Peak Pro). Portable spirometers have been used as a clinical management tool (24), but the current device has not previously been used as a measure of disease control in a clinical trial. It is generally recognized that there is the potential for patients to comply poorly with measurement of airway function and dosing of medication (25, 26) and that their compliance is improved if they are aware that electronic devices monitor the time of measurement or dosing (27, 28).

This study was not designed to determine the adverse event profile of ciclesonide and we did not measure systemic adverse effects, although previous studies have failed to demonstrate an effect of ciclesonide on measurements of the hypothalamic–pituitary–adrenal axis at clinically effective doses (9, 10, 29). However no patient had oral candida or voice change and the incident of sore throat lasted for 1 day. Sputum eosinophil count, a secondary endpoint, was analysed in a subgroup of patients. Other subjects were not enrolled to provide induced sputum samples, did not have sputum eosinophilia at baseline, or were not able to expectorate evaluable sputum samples at all visits. Although these subjects had a higher FEV_1, there was no significant difference in terms age or hyperresponsiveness to AMP or methacholine between those providing or not providing a sputum sample. We do not believe that the difference in FEV_1 between the groups alters the conclusions of our study as FEV_1, was not a primary endpoint and we are not making any comparisons in terms of treatment response between patients with and without sputum induction.

In conclusion, we showed that once daily low dose (160 μg) inhaled ciclesonide demonstrated significant improvements in terms of measures of airway inflammation. These data are in keeping with previous data showing improvement in patients' symptoms and pulmonary function at this once daily dose and anti-inflammatory effects at higher doses. It is important to demonstrate anti-inflammatory effects at 160 μg once daily as this is the current licensed dose of ciclesonide for the treatment of patients with mild to moderate asthma.

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