

Effects of ciclesonide and fluticasone propionate on allergen-induced airway inflammation and remodeling features

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Background: Several topical corticosteroids are available as anti-inflammatory treatment for asthma. Their comparative effects on allergic inflammation and airway remodeling are unclear.

Objective: We compared the effects of ciclesonide with those of fluticasone propionate in a Brown Norway rat model of chronic allergic asthma.

Methods: Rats sensitized and exposed to ovalbumin (OVA) were treated with dry powder vehicle, ciclesonide, or fluticasone (0.01, 0.03, and 0.1 mg/kg administered intratracheally) 24 hours and 1 hour before each of 6 OVA exposures. In a second protocol we administered 0.1 mg/kg ciclesonide or fluticasone only after the third OVA exposure. **Results:** Ciclesonide at all doses inhibited the allergen-induced increase in airway eosinophils and T cells, reduced goblet cell hyperplasia, and decreased 5-bromo-2'-deoxyuridine-immunoreactive airway smooth muscle (ASM) and epithelial cells. At 0.03 and 0.1 mg/kg ciclesonide, bronchial hyper-responsiveness (BHR) was also inhibited. Fluticasone did not attenuate allergen-induced BHR, despite inhibiting airway eosinophils and T cells, goblet cell hyperplasia, and 5-bromo-2'-deoxyuridine-immunoreactive ASM and epithelial cells. Fluticasone (0.1 mg/kg) caused a significant reduction in body weight (9%) compared with ciclesonide (0.1 mg/kg).

Ciclesonide did not change plasma corticosterone levels, whereas fluticasone (0.1 mg/kg) reduced them. In the second protocol both fluticasone and ciclesonide inhibited BHR, bronchial inflammation, goblet cell hyperplasia, and ASM proliferation.

Conclusion: Ciclesonide potently inhibited chronic allergic inflammation, remodeling, and BHR without having an effect

on body weight and the hypothalamic-pituitary-adrenal axis. Fluticasone prevented airway inflammation but not BHR, but both fluticasone and ciclesonide are effective at reversal of BHR, inflammation, and remodeling features. (*J Allergy Clin Immunol* 2005;115:989-96.)

Key words: Bronchial hyperresponsiveness, airway remodeling, airway inflammation, ciclesonide, fluticasone propionate

Asthma is a chronic inflammatory disease of the airways characterized by the presence of activated inflammatory cells, such as eosinophils, mast cells, macrophages, and T lymphocytes, within the bronchial mucosa.^{1,2} There are also changes to structural cells of the airways, including increases in airway smooth muscle (ASM) mass,³ goblet cell hyperplasia,^{4,5} epithelial cell disruption,⁶ and sub-epithelial fibrosis.⁷ ASM cell hyperplasia and hypertrophy might result in thickening of the airway wall,^{8,9} which could contribute to increased nonspecific bronchial hyper-responsiveness (BHR) and to the irreversible airflow obstruction sometimes observed in patients with asthma.¹⁰

Inhaled glucocorticoids, considered the standard therapy for asthma, reduce airway inflammation,^{11,12} BHR,^{12,13} and airway wall remodeling.^{14,15} However, any dose increase in long-term therapy of chronic severe asthma is limited by systemic side effects, such as suppression of hypothalamic-pituitary-adrenal function,^{16,17} growth impairment, disturbed glucose tolerance, or increased risks for osteoporosis and cataract formation.¹⁸ The systemic bioactivity of inhaled glucocorticoids is mainly determined by the systemic absorption from the portion deposited in the airways, and any swallowed part of the dose usually undergoes extensive first-pass hepatic metabolism.¹⁹ The major challenge in developing novel inhaled glucocorticoids is to design substances with high local potency in the airways but with minimal systemic absorption from the airways, thus reducing the risk for systemic side effects.

Ciclesonide, a nonhalogenated inhaled corticosteroid with little oral bioavailability, has been developed for the treatment of asthma.²⁰ Ciclesonide is activated in the lung to a potent active metabolite, desisobutyl-cliclisonide, the affinity of which to the glucocorticoid receptor is about 100 times higher compared with that of its parent, ciclesonide. Ciclesonide was found to be clinically effective in patients with bronchial asthma and caused lower airway responsiveness to adenosine-5'-monophosphate.²¹ The active metabolite has a minor potential for systemic side effects because it is lipophilic and readily

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Abbreviations used

ASM: Airway smooth muscle
BHR: Bronchial hyperresponsiveness
MBP: Major basic protein
OVA: Ovalbumin

conjugates to fatty acids at a hydroxyl group available at C21. It persists longer in the lung, and any absorbed portion is highly bound to plasma proteins, hence minimizing the free fraction in the circulation.

We investigated the anti-inflammatory effects of ciclesonide on chronic allergen-induced airway asthmatic responses in the Brown Norway (BN) rat model of chronic inflammation in 2 different protocols: one to determine the prophylactic effects of topical ciclesonide on the effects of chronic allergen exposure and the other to examine the reversal effects of the corticosteroid on established inflammation and airway remodeling. We focused on the effects of ciclesonide on ASM and epithelial cell DNA synthesis and on chronic eosinophilic inflammation after repeated allergen exposure in BN rats actively sensitized to ovalbumin (OVA). We monitored body weight changes and plasma corticosterone levels as markers of systemic side effects. The effects of ciclesonide were compared with those of fluticasone propionate, an established inhaled corticosteroid for the treatment of asthma.

METHODS**Study design**

Pathogen-free male BN rats (220–250 g; Harlan, Bicester, United Kingdom) were sensitized on days 1, 2, and 3 with 1 mg/kg (intraperitoneal) injections of OVA with 100 mg of Al(OH)₃. On days 6, 9, 12, 15, 18, and 21, animals were exposed to either saline or 1% OVA aerosol for 20 minutes.²² Ciclesonide or fluticasone (made to 10 mg of powder) was instilled intratracheally.

Protocol 1. We studied the following groups:

1. Sensitized, vehicle treated, and repeatedly saline exposed (saline group, n = 8): Animals received α -lactose monohydrate dry powder (Respitose; DMV International Pharma, Veghel, The Netherlands) as vehicle intratracheally 24 hours and 1 hour before each saline exposure.
2. Sensitized, vehicle treated, and repeatedly OVA exposed (OVA group, n = 8): The procedures were the same as for the saline group, except the aerosol was 1% OVA aerosol.
3. Sensitized, ciclesonide treated, and repeatedly OVA exposed (ciclesonide group, n = 8): The procedures were the same as for the OVA group. Rats received either 0.01, 0.03, or 0.1 mg/kg ciclesonide 24 hours and 1 hour before each OVA exposure.
4. Sensitized, fluticasone treated, and repeatedly OVA exposed (fluticasone group, n = 8): The procedures were the same as for group OVA. Rats received either 0.01, 0.03, or 0.1 mg/kg fluticasone 24 hours and 1 hour before each OVA exposure.

Protocol 2.

1. Sensitized, vehicle treated, and repeatedly saline exposed (saline group, n = 6): Animals received α -lactose monohydrate dry powder as vehicle intratracheally 24 hours and 1 hour before saline exposure on days 15, 18, and 21.

2. Sensitized, vehicle treated, and repeatedly OVA exposed (OVA group, n = 6): The procedures were the same as for the saline group, except the aerosol was 1% OVA aerosol.
3. Sensitized, ciclesonide treated, and repeatedly OVA exposed (ciclesonide group, n = 8): The procedures were the same as for the OVA group. Rats received 0.1 mg/kg ciclesonide 24 hours and 1 hour before OVA exposure on days 15, 18, and 21.
4. Sensitized, fluticasone treated, and repeatedly OVA exposed (fluticasone group, n = 8): The procedures were the same as for the OVA group. Rats received 0.1 mg/kg fluticasone 24 hours and 1 hour before OVA exposure on days 15, 18, and 21.

Measurement of bronchial responsiveness

Twenty-four hours after the last OVA exposure, lung resistance was measured with whole-body plethysmography (EMMS, Hants, United Kingdom)²² after achievement of anesthesia with 0.3 mL/kg Hypnorm (administered intramuscularly) in response to concentrations of acetylcholine aerosol. Acetylcholine responsiveness was measured as the concentration of acetylcholine that increased baseline resistance by 100% to 200% (PC₁₀₀ or PC₂₀₀).

Bromodeoxyuridine immunohistochemistry

5-Bromo-2'-deoxyuridine (BrdU; Sigma Chemicals, Poole, United Kingdom; 50 mg/kg administered intraperitoneally) was injected after each allergen exposure, with a second dose 8 to 12 hours later. Briefly, cryostat lung sections were incubated with a primary anti-BrdU mAb solution (clone BU-1; Amersham International, Buckinghamshire, United Kingdom),^{22,23} and BrdU-positive cells were visualized with 3, 3'-diaminobenzidine tetrahydrochloride solution (Sigma) with glucose oxidase–nickel enhancement to produce a black end product.²⁴ α -Smooth muscle actin was detected with a primary anti- α -smooth muscle actin, mAb (clone 1A4, Sigma) by using the alkaline phosphatase–antialkaline phosphatase method. Nuclei were counterstained with the fluorescent DNA ligand 4,6-diamidino-2-phenylindole hydrochloride (Sigma). The number of BrdU-immunoreactive nuclei divided by the total number of nuclei (BrdU plus 4,6-diamidino-2-phenylindole hydrochloride nuclei) within the α -smooth muscle actin defined the immunoreactive area. Epithelial DNA synthesis was measured as the number of BrdU-positive cells per unit length of the epithelium defined by a basement membrane mask.²²

Eosinophil, T-cell, and goblet cell counts

We used an IgG1 mAb against human major basic protein (MBP), clone BMK-13 (Monosan, Uden, The Netherlands), and a mouse anti-rat CD2 mAb (pan-T-cell markers; PharMingen, Cambridge Bioscience, Cambridge, United Kingdom). Positively stained cells were visualized with the alkaline phosphatase–antialkaline phosphatase method. Positively stained cells in the bronchial submucosa around the 5 largest airways were measured per millimeter of basement membrane. By using Alcian blue/periodic acid–Schiff staining, the mean number of periodic acid–Schiff-positive goblet cells was determined.

Plasma corticosterone level

Plasma corticosterone levels were determined by means of RIA. Calibration was done with standards of corticosterone supplied with the kit covering a range of 57.7 to 5772 nmol/L.

Data analysis

Data are presented as means $\pm$ SEM. For comparisons of different groups, the Kruskal–Wallis test for ANOVA was used. If the result of

the Kruskal-Wallis test for ANOVA was significant, we applied the Mann-Whitney *U* test for comparison between 2 individual groups. *P* values of less than .05 were considered significant.

RESULTS

Protocol 1

Weight. Body weight changes were calculated as the percentage of weight change from day 1, the starting day, to day 22, the lung function day. After repeated allergen exposure, the ciclesonide treatment group demonstrated no significant reduction in mean body weight, whereas the 0.1 mg/kg fluticasone-treated group showed a reduction in mean body weight ($-9.20\% \pm 0.54\%$, $P < .001$, Fig 1) compared with that seen in the vehicle-exposed group.

Bronchial responsiveness to acetylcholine. In repeated OVA-exposed, vehicle-treated rats, there was an increase in bronchial responsiveness ($-\log PC_{100} = 2.46 \pm 0.17$ [95% CI, 2.05-2.87]) compared with that seen in repeated saline-exposed and vehicle-treated rats (1.91 ± 0.12 [95% CI, 1.62-2.21], $P < .05$). Ciclesonide (0.03 and 0.1 mg/kg) inhibited the increased bronchial responsiveness (1.28 ± 0.13 [95% CI, 0.97-1.59], $P < .001$, and 1.74 ± 0.22 [95% CI, 1.24-2.25], $P < .05$, respectively). Fluticasone at all doses did not inhibit allergen-induced increased bronchial responsiveness (Fig 2, A).

Goblet cell count. Repeated allergen exposure caused an increase in goblet cell numbers in rat airways (17.9 ± 1.67 cell/mm [95% CI, 14.0-21.9 cell/mm] basement membrane vs 9.94 ± 1.69 cell/mm [95% CI, 5.96-13.9 cell/mm], $P < .01$) when compared with that in control animals (Fig 3, A). Ciclesonide (0.01, 0.03, and 0.1 mg/kg) significantly inhibited the allergen-induced increase in goblet cell number (11.0 ± 1.13 [$P < .001$], 11.5 ± 1.21 [$P < .01$], and 10.9 ± 1.01 [$P < .001$], respectively). Fluticasone (0.03 and 0.1 mg/kg) significantly inhibited the allergen-induced increase in goblet cell number (11.8 ± 1.11 [$P < .05$] and 5.38 ± 1.20 [$P < .001$], respectively). Overall, fluticasone (0.1 mg/kg) caused the greatest reduction in goblet cell numbers.

ASM and epithelial BrdU indices. After repeated allergen challenge, there was an increase in the percentage of ASM cells expressing BrdU in the allergen-exposed and vehicle-treated group ($3.05\% \pm 0.20\%$ [CI, 2.57-3.52]) compared with that seen in the sensitized, saline-exposed, and vehicle-treated groups ($1.11\% \pm 0.10\%$ [CI, 0.86-1.35], $P < .001$). Both ciclesonide and fluticasone at all doses significantly attenuated ASM cell DNA synthesis after repeated allergen exposure (Fig 4, A). Similarly, repeated allergen exposure caused an increase in the number of BrdU-positive epithelial cells per millimeter of basement membrane in the allergen-exposed and vehicle-treated group of rats (33.8 ± 3.77 [CI, 7.81-22.3]) compared with that seen in the saline-exposed and vehicle-treated control animals (15.10 ± 3.07 [CI, 7.81-22.3], $P < .001$). Ciclesonide and fluticasone at all doses attenuated the epithelial cell DNA synthesis after repeated allergen exposure (Fig 4, B). At the highest dose of fluticasone, there was suppression of BrdU-positive cells

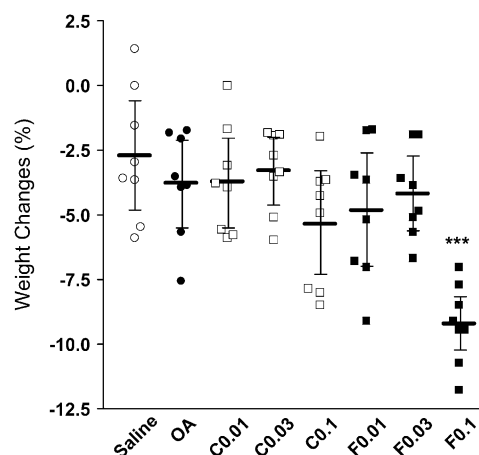


FIG 1. Weight loss in different groups after sensitization, 6 exposures to OVA, and 12 treatments with increasing doses of ciclesonide or fluticasone or after carrier from protocol 1 (***) $P < .001$ compared with the saline group). Data are shown as means and 95% CI. OA, Ovalbumin; C0.01, ciclesonide 0.01 mg/kg; C0.03, ciclesonide 0.03 mg/kg; C0.1, ciclesonide 0.1 mg/kg; F0.01, fluticasone 0.01 mg/kg; F0.03, fluticasone 0.03 mg/kg; F0.1, fluticasone 0.1 mg/kg.

in the epithelium to less than the baseline values of saline-exposed rats.

Airway inflammation. Repeated allergen exposure caused an increase in MBP-positive cells (32.7 ± 6.42 cell/mm basement membrane vs 17.7 ± 2.49 , $P < .05$) and CD2-positive T cells (34.5 ± 5.30 vs 19.0 ± 1.15 , $P < .01$) when compared with control values (Fig 5). Ciclesonide and fluticasone at all doses inhibited the allergen-induced increase in MBP-positive cells and CD2-positive T cells (Fig 5). At the highest dose of fluticasone, MBP-positive eosinophils and CD2-positive T cells were less than the baseline values of saline-exposed rats.

Plasma corticosterone level. After repeated allergen challenge, there was no change in plasma corticosterone level in the OVA-exposed and vehicle-treated group compared with that seen in the saline-exposed and vehicle-treated group (1120 ± 73 nmol/L [95% CI, 945-1290 nmol/L]). Ciclesonide at all doses did not affect plasma corticosterone levels. Treatment with 0.1 mg/kg fluticasone significantly suppressed plasma corticosterone levels (948 ± 51 nmol/L [CI, 827-1070 nmol/L], $P < .05$) compared with those seen in the OVA-exposed and vehicle-treated group.

Protocol 2

There were no significant differences in body weights between the groups. In this protocol there was also an increase in bronchial responsiveness after chronic allergen exposure, with $-\log PC_{200}$ increasing from 1.37 ± 0.12 to 1.73 ± 0.07 ($P < .05$). Both ciclesonide and fluticasone inhibited this increase in bronchial responsiveness (Fig 2). In addition, the increase in eosinophil and lymphocyte counts in lavage fluid after chronic allergen challenge was inhibited by fluticasone, but with

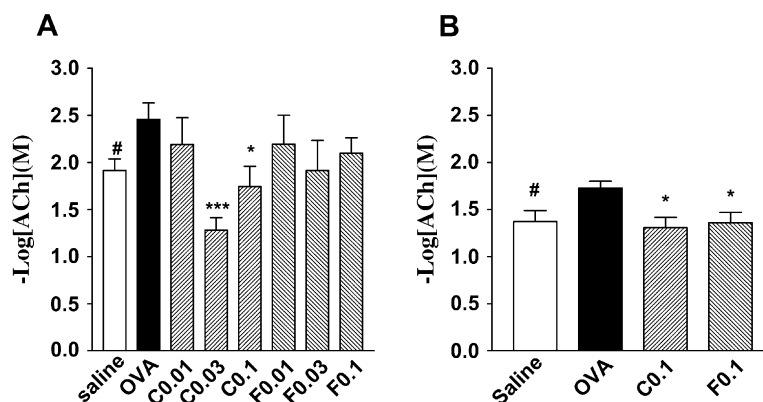


FIG 2. Effects of ciclesonide and fluticasone on bronchial responsiveness to acetylcholine. **A** shows the results from protocol 1, and **B** shows those from protocol 2 ($^{\#}P < .05$ compared with the saline group; $^*P < .05$ and $^{***}P < .001$ compared with the OVA group). Data are shown as means $\pm$ SEM. OVA, Ovalbumin; C0.01, ciclesonide 0.01 mg/kg; C0.03, ciclesonide 0.03 mg/kg; C0.1, ciclesonide 0.1 mg/kg; F0.01, fluticasone 0.01 mg/kg; F0.03, fluticasone 0.03 mg/kg; F0.1, fluticasone 0.1 mg/kg.

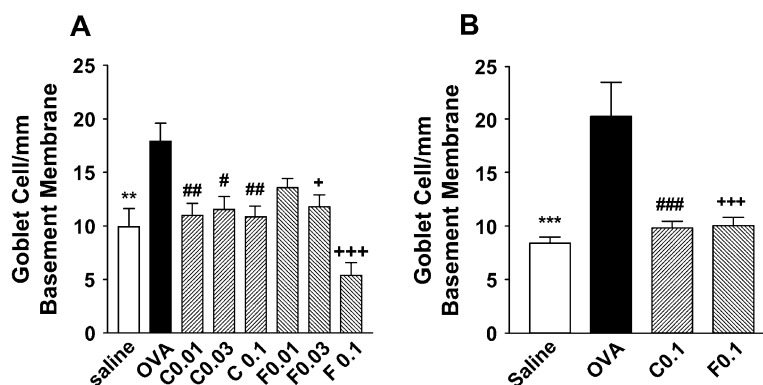


FIG 3. Effect of inhaled ciclesonide and fluticasone on goblet cell hyperplasia in the airways. **A** shows the results from protocol 1, and **B** shows those from protocol 2 ($^{**}P < .01$ compared with the saline group; $^{\#}P < .05$, $^{##}P < .01$, $^{###}P < .001$, $^{+}P < .05$, and $^{+++}P < .001$ compared with the OVA group). OVA, Ovalbumin; C0.01, ciclesonide 0.01 mg/kg; C0.03, ciclesonide 0.03 mg/kg; C0.1, ciclesonide 0.1 mg/kg; F0.01, fluticasone 0.01 mg/kg; F0.03, fluticasone 0.03 mg/kg; F0.1, fluticasone 0.1 mg/kg.

ciclesonide, the reduction in eosinophil and lymphocyte counts was not significant (Fig 6). However, total cell recovery was significantly inhibited by both ciclesonide and fluticasone. There was no effect of either treatment on the induced increase in neutrophil counts. Submucosal counts of eosinophils and CD2-positive T cells were significantly increased after chronic allergen exposure, but this increase was suppressed by both ciclesonide and fluticasone (Fig 5). Both ciclesonide and fluticasone inhibited allergen-induced goblet cell hyperplasia (Fig 3, B) and incorporation of BrdU in epithelium and ASM cells (Fig 4).

DISCUSSION

We investigated the effects of ciclesonide, a novel inhaled corticosteroid that is converted locally in the lung to a potent active metabolite, in preventing allergic

inflammation, BHR, and airway remodeling induced after repeated exposures to allergen. Ciclesonide, at the same inhaled dose as fluticasone, demonstrated a comparable potency in inhibiting airway inflammation and remodeling but without producing the systemic effects, such as weight loss and plasma corticosterone reduction, as fluticasone at the same dose ranges used. In addition, it prevented BHR induced by chronic allergen exposure, whereas fluticasone did not. To determine whether these topical corticosteroids could also reverse the established effects of chronic allergen exposure, we administered them after 3 exposures to allergen. We found that at the dose of 0.1 mg/kg, both ciclesonide and fluticasone were effective at reversing BHR and inflammatory changes. These results indicate that ciclesonide is effective at preventing and reversing BHR, whereas fluticasone is less effective at preventing allergen-induced BHR.

The rat model has been used for studies of airway inflammation, BHR, and airway remodeling.^{14,15,25-27}

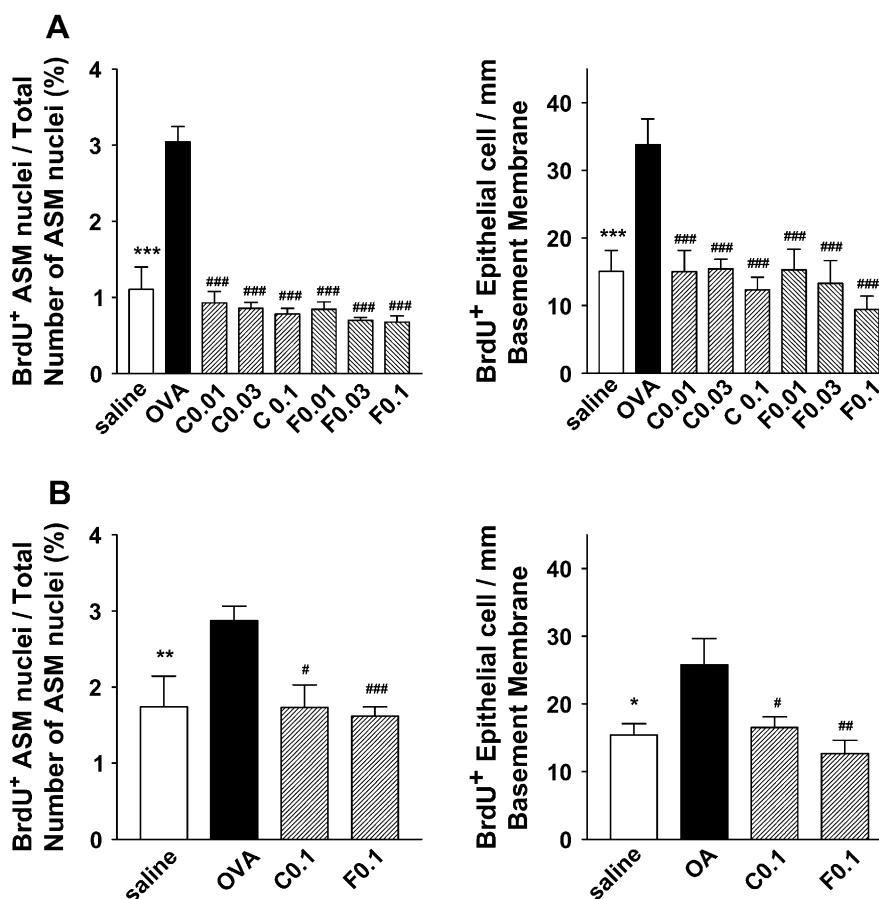


FIG 4. Effects of ciclesonide and fluticasone on ASM and epithelial cell BrdU incorporation after repeated allergen challenge. **A** shows the results from protocol 1, and **B** shows those from protocol 2 (*** $P < .001$ compared with the saline group; ### $P < .001$ compared with the OVA group; mean $\pm$ SEM). ASM, Airway smooth muscle; BrdU, bromodeoxyuridine; OVA or OA, ovalbumin; C0.01, ciclesonide 0.01 mg/kg; C0.03, ciclesonide 0.03 mg/kg; C0.1, ciclesonide 0.1 mg/kg; F0.01, fluticasone 0.01 mg/kg; F0.03, fluticasone 0.03 mg/kg; F0.1, fluticasone 0.1 mg/kg.

Repeated allergen exposure over 3 weeks led to an increase in airway inflammatory cell infiltration with eosinophils and T cells and increased proliferation of ASM and epithelium accompanied by an increased BHR.^{22,28} In the present study intratracheal instillation of ciclesonide 24 hours and 1 hour before chronic allergen exposure effectively inhibited the allergen-induced increase in eosinophils and T cells infiltrating the airways, reduced airway goblet cell hyperplasia, and decreased ASM and epithelial cell proliferation. At doses of 0.03 and 0.1 mg/kg, ciclesonide also attenuated allergen-induced BHR, although the greatest inhibition was found at the 0.03 mg/kg dose. The explanation for this is unclear, although one possibility might be related to the non-significant weight loss of the rats after treatment with the higher dose of ciclesonide. Fluticasone resulted in similar inhibitory effects on cellular inflammation and proliferation as observed with ciclesonide but was without effect on BHR. Fluticasone caused a significant reduction in body weight and suppression of plasma cortisol levels at the dose of 0.1 mg/kg, when the greatest inhibition of goblet

cell hyperplasia and eosinophilic inflammation was observed.

Previous studies of ciclesonide in the BN rat model have examined the effect of single doses. Airway eosinophilia induced by single allergen exposure was inhibited by a single intratracheal administration of ciclesonide with a dose needed to cause 50% inhibition of 0.5 mg/kg.²⁹ In a comparative study of the effects of single intratracheal doses of ciclesonide and fluticasone, ciclesonide was 7.2- to 7.9-fold less potent than fluticasone in inhibiting eosinophilia, but it was also 24-fold less active than fluticasone in inducing femoral growth plate proliferation.³⁰ In the present study we studied the chronic effect of topical corticosteroid use in a chronic model of allergen challenge on BHR, airway inflammation, and airway remodeling effects because inhaled corticosteroid is used as a chronic treatment in asthmatic patients. We used a lower intratracheal dose of ciclesonide or fluticasone than that used in other single-dose studies because in preliminary studies we found that doses in excess of 100 μ g/kg (given 12 times over 3 weeks) caused a significant

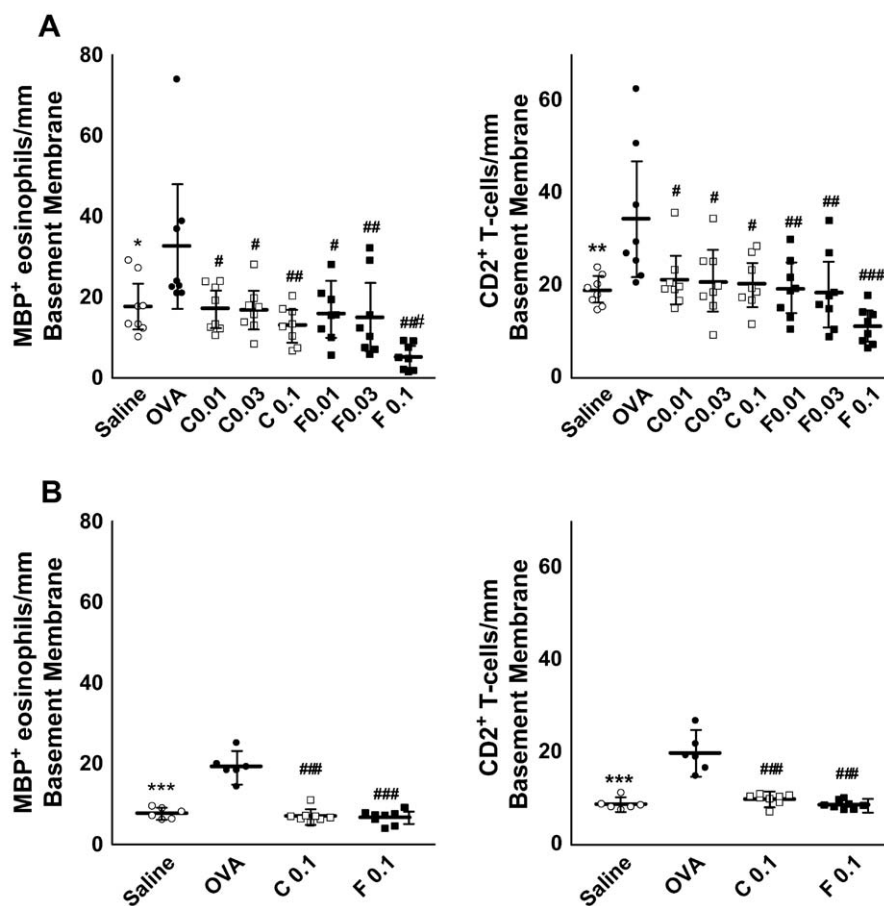


FIG 5. Effects of ciclesonide on MBP-positive eosinophil (A) and CD2-positive T-cell (B) recruitment into the airways after repeated allergen challenge. Fig 5, A, shows the results from protocol 1, and Fig 5, B, shows those from protocol 2 (* $P < .05$ and ** $P < .01$ compared with the saline group; # $P < .05$, ### $P < .01$, and ### $P < .001$ compared with the OVA group; mean $\pm$ 95% CI). MBP, Major basic protein; OVA, ovalbumin; C0.01, ciclesonide 0.01 mg/kg; C0.03, ciclesonide 0.03 mg/kg; C0.1, ciclesonide 0.1 mg/kg; F0.01, fluticasone 0.01 mg/kg; F0.03, fluticasone 0.03 mg/kg; F0.1, fluticasone 0.1 mg/kg.

reduction in body weight. The dose range we used in the present study is close to that used clinically when based on a microgram per kilogram body weight dosing. With regard to tissue eosinophilia, we found equivalent degrees of inhibition at the 0.01 and 0.03 mg/kg doses of ciclesonide and fluticasone, but a much greater inhibition was observed at 0.1 mg/kg fluticasone. Similar results were observed for inhibition of CD2 T cells and of goblet cell hyperplasia. However, BHR was not significantly inhibited by fluticasone, whereas inhibition was observed with ciclesonide (0.03 and 0.1 mg/kg). As a measure of airway wall remodeling, we used indices of ASM and epithelial cell proliferation and found significant inhibition at all doses of fluticasone and ciclesonide, indicating that these parameters were more sensitive to topical corticosteroids than BHR. Our study also indicates that for chronic dosing rather than single dosing, a smaller dose of corticosteroid is needed.

BHR might result from an increase in airway wall thickness as a result of remodeling.³¹ However, we have previously not been able to demonstrate changes in airway

wall thickness, despite the presence of persistent airway inflammation and ASM cell proliferation.^{28,32} In addition, inhibition of allergic inflammation might not be associated with a reduction in BHR, indicating that the latter might be independent of airway inflammation.^{14,15} Other studies in the BN rat model have shown that in established remodeling, inhaled corticosteroids can protect the airway from further progression of structural changes but might not reverse it.^{15,33} A higher dose of inhaled corticosteroid was needed to inhibit BHR than to reduce airway eosinophilia, and therefore BHR is less sensitive to inhaled corticosteroids than the eosinophilic response.

It is possible that the discrepancy in the inhibitory effect on allergen-induced BHR between ciclesonide and fluticasone might be related to the property of ciclesonide in being converted to a more potent metabolite locally in the airways, thus providing a higher effective concentration of corticosteroids locally in the airways. For most inhaled corticosteroids, usually only a small percentage of drugs is retained in the lung, whereas a large portion is swallowed and absorbed. Thus at the same doses as ciclesonide,

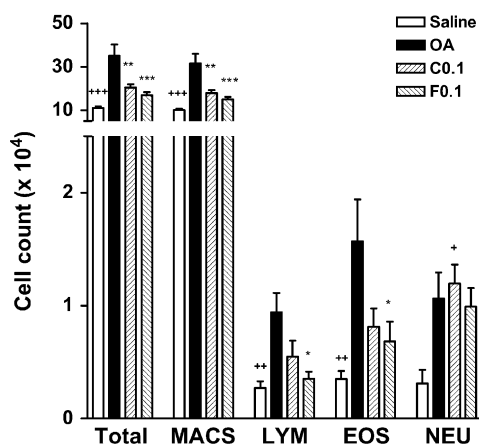


FIG 6. Differential cell count of bronchoalveolar lavage fluid from protocol 2 ($+P < .05$, $++P < .01$, and $+++P < .001$ compared with the saline group; $*P < .05$, $**P < .01$, and $***P < .001$ compared with the OVA group; mean $\pm$ SEM). OA, ovalbumin; C0.1, ciclesonide 0.1 mg/kg; F0.1, fluticasone 0.1 mg/kg; MACS, macrophages; LYM, lymphocytes; EOS, eosinophils; NEU, neutrophils.

fluticasone inhibits airway wall inflammation but had no effect on BHR, as seen with ciclesonide. The profound reduction in goblet cells, eosinophils, T cells, and plasma corticosterone levels to a level lower than baseline at the highest dose of fluticasone (0.1 mg/kg) is of interest. This is also coincident with the 9% decrease in body weight as found with the 12 doses of fluticasone at 0.1 mg/kg in protocol 1. However, there was no significant effect on body weight in protocol 2, when half this dose was administered. It is not excluded that these changes might be related to generalized weight change and are closely related to bone marrow and hormone suppression.^{34,35} Corticosteroids have regulatory effects on ASM proliferation induced by mitogens.³⁶ Suppression of ASM proliferation at less than the baseline level, even at 0.01 mg/kg of both steroids, indicates that ASM proliferation is more susceptible and sensitive to steroid regulation compared with other parameters. The mechanism is still not clear.

In a study of fluticasone on allergen-induced airway responses in asthmatic patients, a single dose before allergen challenge was shown to be effective in inhibiting the allergen-induced late phase and BHR, whereas 2 weeks of treatment with fluticasone did not significantly inhibit these parameters.³⁷ Ciclesonide administered for 2 weeks before allergen challenge inhibited both the early- and late-phase responses in patients with mild asthma,³⁸ but bronchial responsiveness was not measured in these studies. The results of these studies are in line with our current findings in the rat. The relative lack of effect of fluticasone in comparison with ciclesonide on this aspect of BHR remains unclear.

In conclusion, ciclesonide has potent inhibitory effects on features of chronic allergic pulmonary inflammation, airway remodeling, and BHR at doses that did not change body weight and hypothalamic-pituitary-adrenal axis. Fluticasone inhibits airway inflammation and airway

wall remodeling and is better at reversing rather than preventing BHR, but it might lead to more systemic side effects at the higher dose.

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