In Vitro and in Vivo Anti-Inflammatory Activity of the New Glucocorticoid Ciclesonide

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ABSTRACT

The glucocorticoid ciclesonide is the 2’R-epimer of 2’-cyclohexyl-11β-hydroxy-21-isobutyroxy-16βH-dioxol[5’,4’:16,17]pregna-1,4-diene-3,20-dione. The active metabolite desisobutyrylciclesonide (des-CIC) is derived from ciclesonide by esterase cleavage of isobutyrate at the C21 position. The relative binding affinities at the rat glucocorticoid receptor were dexamethasone, 100; ciclesonide, 12; des-CIC, 1212; and budesonide, 905. Des-CIC potently inhibited the activation of murine and human lymphocytes in a series of different in vitro systems. With the exception of concanavalin A-stimulated rat spleen cells, des-CIC was more potent than the parent compound. Des-CIC compared well with budesonide in all in vitro systems. Furthermore, the respective 2’S-epimers were always significantly less potent than the 2’R-epimers. In vivo, ciclesonide (intratracheal administration), des-CIC, and budesonide inhibited antigen-induced accumulation of eosinophils, protein, and tumor necrosis factor-α into the bronchoalveolar lavage fluid of ovalbumin-sensitized and -challenged Brown Norway rats with an ED50 value ranging from 0.4 to 1.3 mg/kg, indicating similar potency, which suggests in vivo activation of the parent compound. Ciclesonide and budesonide inhibited the bradykinin-induced protein leakage into the rat trachea. In the rat cotton pellet model, ciclesonide inhibited granuloma formation (ED50 = of 2 μg/pellet), whereas budesonide and des-CIC were 15- and 20-fold less active; thymus involution was induced with an ED50 of 303, 279, and 154 μg/pellet, respectively. When applied orally to rats for 28 days, ciclesonide showed low potency in reducing weight of thymus and adrenals, suggesting low oral bioavailability. The in vivo data on ciclesonide highlight its effective local action and a reduced potential for side effects.

Allergic diseases of the upper and lower airways, rhinitis, and asthma are the most common respiratory disorders in industrialized countries (Gienbycz, 2000; Skoner, 2001). Corticosteroids represent the most effective anti-inflammatory treatment for these diseases (Barnes, 1995; Bachert and Gevert, 1999). For asthma, corticosteroids have become the standard anti-inflammatory treatment because long-term treatment with inhaled steroids reduces the number of inflammatory cells, specifically the mast cells, eosinophils, and lymphocytes, in the airway mucosa and submucosa, leading to clinical improvement (Burke et al., 1992; Bentley et al., 1996; Skoner, 2001). However, long-term treatment with steroids is associated with systemic side effects that, even with inhaled administration, include possible growth retardation in children, osteoporosis, ocular complications, and infections in the oropharynx (Toogood, 1998). Due to such side effects of inhaled or intranasal corticosteroids, there is a need to improve the existing glucocorticoids.

Several strategies have been followed to develop anti-inflammatory steroids with diminished side effects. “Dissociated” corticosteroids favor the concept that the anti-inflammatory effects of glucocorticoids may be mediated by repression of transcription factors for proinflammatory mediators (transrepression), whereas the endocrine and metabolic effects of glucocorticoids may be mediated through binding to glucocorticoid response elements (Adcock, 2001). The clinical relevance of this concept is still unclear and has been challenged recently (Belvisi et al., 2001). Another approach under investigation was the development of “soft” steroids (e.g., loteprednol etabonate). Such steroids are synthesized from inactive metabolites and, in theory,
should yield highly active but metabolically unstable drugs (Bodor, 1988; Szelenyi et al., 2000).

The development of ciclesonide, however, followed a different concept because ciclesonide is activated on-site by esterases in the lung tissue. Ciclesonide has very low affinity for the glucocorticoid receptor, whereas in the target tissue it is readily converted into an active metabolite (desisobutyril-ciclesonide; des-CIC) (Mealy et al., 2001). This activation occurs by ester cleavage at the C21 position of ciclesonide. The affinity of des-CIC to the glucocorticoid receptor is about 100-fold higher than that of ciclesonide. Ciclesonide is the pure 2'R-epimer of 2'-cyclohexyl-11β-hydroxy-21-isobuturyloxyl-16βH-dioxolo[5',4':16,17]pregna-1,4-diene-3,20-dione (CAS RN: 141845-81-0).

BYK20426: ciclesonide (INN), the 2'R-epimer of 2'-cyclohexyl-11β-hydroxy-21-isobuturyloxy-16βH-dioxolo[5',4':16,17]pregna-1,4-diene-3,20-dione (Fig. 1).


Fig. 1. Structure of ciclesonide, des-CIC, and the respective 2'S-epimers.

Binding to the Rat Lung Glucocorticoid Receptor

Male adrenolecetomized Sprague-Dawley rats (150–200 g) from Harlan (Indianapolis, IN) were used for the production of lung cytosol 6 days after adrenalectomy. Cytosol was prepared with slight modifications as described previously (Rohdewald et al., 1984). Briefly, the frozen tissue was crushed and further homogenized after addition of 6 volumes of ice-cold incubation buffer (10 mM Tris/HCl, 10 mM sodium molybdate, 2 mM 1,4-dithiothreitol) using a Virtus homogenizer (four periods of 45 s each, full speed, with a 1-min cooling period between every step). The homogenate was centrifuged for 1 h at 4°C at 105,000g. Aliquots of the aqueous supernatant (cytosol) were frozen in liquid nitrogen and stored at −80°C. Frozen cytosol was thawed and diluted with 2 to 6 volumes of incubation buffer that contained corticenic acid (final concentration of 10−5 M) sufficient to block the transcortin binding sites, a saturable glucocorticoid binding protein present in the blood. Corticenic acid has been shown to have no binding activity to the triamcinolone binder in rat lung cortisol, but has high affinity to transcortin (Manz et al., 1982). Portions of cytosol (160 µl) were incubated with 20 µl of [3H]triamcinolone acetonide (final concentration in the incubation mixture, 15 nM) and the same volume of varying concentrations of competitor (in ethanol). After 24 h at 4°C, unbound steroid was removed with 400 µl of activated charcoal suspension [2% (w/v), in incubation buffer]. After further incubation for 10 min, the suspension was centrifuged and the radioactivity in 400 µl of supernatant was determined. In a single competition experiment, competition behavior of two to three steroids was compared with that of the standard competitor dexamethasone. To block nonspecific esterase activity, PMSF or DFP was added to the cytosol. Usually, PMSF (final concentration, 0.6 mM) was added to the cytosol to assess the affinity of des-CIC and BYK20433 (two independent experiments) and of ciclesonide, BYK20427, and budesonide (one experiment). In another experiment that dealt with the determination of the activity of ciclesonide, BYK20427, and budesonide, incubations were performed with DFP (final concentration, 15 mM). This inhibitor was used to verify the activity of PMSF in blocking the enzymatic activation of ciclesonide in the incubation mixture. In all experiments, dexamethasone served as reference competitor and was assigned a relative binding affinity of 100. Nonspecific binding was determined in the presence of 10−6 M unlabeled dexamethasone. All incubations were performed in duplicate. The IC50 values of a given steroid (competitor necessary to displace 50% of the specific [3H]triamcinolone acetonide binding) were determined in two independent experiments performed on separate days. The IC50 values of the investigated steroids were determined by a nonlinear curve-fitting procedure and were transformed into relative binding affinities (RBAs) using dexamethasone (RBA = 100) as a reference substance: RBAunknown = 100×IC50competitor/IC50dexamethasone.

Materials and Methods

Nomenclature

BYK20426: ciclesonide (INN), the 2'R-epimer of 2'-cyclohexyl-11β-hydroxy-21-isobuturyloxy-16βH-dioxolo[5',4':16,17]pregna-1,4-diene-3,20-dione (CAS RN: 126544-47-6) (Fig. 1).


Inhibition of ConA-Induced Proliferation of Rat Spleen Cells

Spleen cells were obtained from Sprague-Dawley rats (Charles River Wiga, Sulzfeld, Germany) after dissection of spleen, passage of cells through a cell strainer (Falcon Plastics, Oxnard, CA), and subsequent centrifugation of cells on a Lymphoprep gradient. Steroids were dissolved at 4 × 10^{-8} M in DMSO; further dilutions were done in complete medium. Medium was RPMI 1640 supplemented with 2 mM glucose, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM pyruvate, 5 × 10^{-5} M 2-mercaptoethanol, 1% (v/v) minimal essential medium nonessential amino acids, and 5% (v/v) FCS. Media and supplements were from Invitrogen (Karlsruhe, Germany). Samples were set up in triplicates. Cells, ConA (Sigma Chemie, Deisenhofen, Germany), and steroid were pipetted into flat-bottom microtiter plates so that the final conditions were volume, 0.2 ml; ConA, 2.5 μg/ml; cells, 1 × 10^{6}/ml; and FCS, 5%, with final concentrations of steroids ranging from 10^{-9} to 10^{-11} M. In this assay, the maximum concentration of DMSO was 0.25%. Control cultures did not contain steroid and were set up as follows: cells + ConA + 0.25% DMSO, cells + ConA + 0.25% DMSO, cells alone. Cultures were incubated for 72 h at 5% CO_2 and 37°C. An MTT assay was performed; 10 μl of MTT (Sigma Chemie) stock solution (5 mg/ml) was added to every well, and the microtiter plates were incubated for further 4 h. Then, 150 μl of supernatant was replaced by DMSO, and the microtiter plates were placed on an orbital shaker for 45 min. The optical density was determined at 540/690 nm using an EL311 microtiter plate reader (Bio-Tek Instruments, Winooski, VT). The IC_{50} values and 95% confidence limits were calculated after nonlinear regression analysis of the concentration-response curve. The IC_{50} values refer to the maximum inhibition achieved in the respective experiments, which was set at 100%. Three independent experiments were performed.

Inhibition of CD3-Induced Release of IL-4 and IL-5 from murine T_{h}2-Lymphocytes (D10.G4.1)

Cells were cultured in RPMI 1640 medium containing HEPES (Roche Diagnostics, Mannheim, Germany), supplemented with 2 mM glucose, 5 × 10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS (Roche Diagnostics). The murine T_{h}2 clone D10.G4.1 (American Type Culture Collection, Manassas, VA) was maintained in culture by stimulation every 10 days with its antigen conalbumin, 5% Rat T-STIM (Collaborative Research, Bedford, MA) as a source of lymphokines and mitomycin C-treated, syngenic (H-2k) splenocytes as antigen-presenting cells. For individual experiments, cells were thawed and cultured for 3 d in complete medium with Rat T-STIM. For the production of interleukin-containing supernatants, cells were placed in 96-well microtiter plates (4 × 10^{4} cells/well/100 μl) for 3.5 h in complete medium without Rat T-STIM. For the production of interleukin-containing supernatants, cells were placed in 96-well microtiter plates (4 × 10^{4} cells/well/100 μl) for 3.5 h in complete medium without Rat T-STIM. Steroids were dissolved in DMSO at 1000 times the final concentration; subsequent dilutions were made in complete medium. Cultures were stimulated by addition of 50 μl of CD3 mAb (hamster anti-mouse CD3 mAb, clone 145-2C11; Cedarlane Laboratories, Hornby, ON, Canada) at a final dilution of 1:100. Two independent experiments were performed with each sample set up in duplicate. Supernatants were harvested after 16 h and stored at −70°C. IL-4 and IL-5 were determined using commercially available ELISA kits (Endogen, Boston, MA) for murine IL-4 and IL-5 according to the manufacturer's protocol. The IC_{50} values were calculated from concentration-inhibition curves by linear regression analysis.

Inhibition of ConA-Induced Proliferation of Human Peripheral Blood Mononuclear Cells (PBMCs)

Preparation of Cells. Heparinized blood (50–100 ml) was obtained from volunteers and diluted 1:2 with RPMI 1640 medium. The cell suspension was layered on top of Lymphoprep and spun at 600g for 20 min. Mononuclear cells were harvested from the interphase of the gradient and diluted 1:5 in medium, centrifuged, and washed three times. Cells were counted in a Neubauer chamber, and viability was assessed using trypan blue. Steroids were dissolved at 4 × 10^{-8} M in 100% DMSO, and further dilutions were done in cell culture medium (RPMI 1640 medium supplemented with 2 mM glucose, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM pyruvate, 5 × 10^{-5} M 2-mercaptoethanol, 1% (v/v) MEM nonessential amino acids, and 10% FCS). Steroid solutions were prepared freshly on the day of experiment.

Set Up of Microtiter Plates and Proliferation Assay. Cells, ConA, and corticosteroids were pipetted into flat-bottom microtiter plates (Costar, Cambridge, MA). The final culture conditions were volume, 0.2 ml; ConA, 0.8 μg/ml; cells, 2 × 10^{4}/ml; and FCS, 10%. Final concentrations of corticosteroids ranged from 10^{-6} M to 10^{-11} M; the maximum concentration of DMSO was 0.25% in this assay. Control cultures did not contain corticosteroid but were set up as follows: cells + ConA + 0.25% DMSO, cells + ConA; cells + 0.25% DMSO, cells alone. Each microtiter plate (one for every corticosteroid) also contained the control cultures described above. All samples were set up in triplicate. Microtiter plates were incubated for 72 h at 37°C, 5% CO_2, 95% humidity and pulsed with 0.5 μCi of [3H]thymidine (specific activity, 2 Ci/mmol) during the last 4 h of culture. Then, the cultures were harvested onto glass fiber strips using a Skatron harvester (Zinsser, Frankfurt, Germany). Incorporation of [3H]thymidine into DNA was assessed using liquid scintillation. In one experiment (experiment 1), proliferation was assessed by determining the incorporation of bromodeoxyxuridine using the bromodeoxyxuridine labeling and detection kit III (Roche Diagnostics) according to the manufacturer’s instructions. Concentration-response curves based on inhibition of ConA-induced [3H]thymidine or bromodeoxyxuridine incorporation were determined using nonlinear regression, and the IC_{50} values and the 95% confidence limits were determined. The IC_{50} values refer to the maximum inhibition achieved in the respective experiments, which was set at 100%.

Inhibition of CD3-Induced Proliferation and Cytokine Production by Purified Human CD4^{+} Lymphocytes

Preparation of Cells. Human CD4^{+} T-lymphocytes were purified from healthy volunteers in a three-step protocol essentially as described previously (Gantner et al., 1997). Citrate-anticoagulated blood was diluted 1:6-fold with PBS before centrifugation for 20 min at 220g. The lower phase was transferred onto a Percoll gradient (p = 1.077 g/ml). After centrifugation at 800g for 25 min at room temperature, the interphase containing the PBMCs was obtained. Cells were washed in PBS and then resuspended in elutriation medium (PBS, 2% fetal bovine serum, 2 mM EDTA, and 5 mM glucose, pH 7.4). Countercurrent elutriation centrifugation was performed using a J2-MC centrifuge equipped with a JE-6B rotor (Beckman Coulter, Fullerton, CA). The fraction containing lymphocytes (>95% purity) was obtained at a flow rate of 32.5 ml/min and a rotor speed of 3000 rpm. Cells were spun down for 5 min at 570g, and resuspended in 10 ml of elutriation medium for a second countercurrent elutriation step at 19 ml/min and 3000 rpm to minimize the contamination of the lymphocyte fraction with platelets. Cells were then resuspended in 700 μl of PBS containing 2% fetal bovine serum and incubated for 1 h at 4°C (shaking) to remove B-cells, monocytes, granulocytes, natural killer cells, and CD8^{+} T-cells. CD4^{+} T-cells were obtained by negative selection of the whole lymphocyte fraction on MACS type CS columns (Miltenyi Biotec, Bergisch Gladbach, Germany) using magnetic antibodies (MACS colloidal superparamagnetic Micro Beads from Miltenyi Biotec) directed against CD19, CD14, CD16, and CD8 (150 μl each). CD4^{+} T-cells obtained under these conditions were >99% pure as determined by cytofluorometric analysis. For the functional studies described below, cells were resuspended in RPMI 1640 medium supplemented with 10% FCS, 2 mM glucose, and 100 U/ml penicillin/100 μg/ml streptomycin.

Stimulation of CD4^{+} T-Cells and Measurement of Proliferation and Cytokine Synthesis. CD4^{+} T-cells were stimulated with
plate-bound CD3 mAb and soluble CD28 mAb. On the day before cell isolation, flat-bottom 96-well microtiter plates (BD Biosciences, Heidelberg, Germany) were coated with 50 µl of CD3 mAb (Orthoclone OKT-3; Janssen-Cilag, Neuss, Germany; 0.5 µg/well in PBS) for about 2.5 h at 37°C and 5% CO₂. Plates were then stored at 4°C overnight and washed three times with PBS before use. From steroid stock solutions (2 mM in DMSO), 1:10 serial dilutions were prepared in DMSO. From each serial dilution, a subsequent intermediate dilution was made (1:100 into complete culture medium containing 2% DMSO), which was finally diluted 1:20 into the assay to produce the desired steroid concentration at 0.15% DMSO. Microtiter plates were set up as follows: 80 µl of culture medium, 10 µl of steroid, 100 µl of CD4+ T-cells (2 × 10⁶ cells/well), and 10 µl of CD28 mAb (clone 28.2; Coulter-Immunotech, Hamburg, Germany; final concentration, 3 µg/ml). Plates were incubated at 37°C, 5% CO₂ for 72 h. For the determination of cytokine synthesis, all assays were performed in quadruplicate. At the end of the incubation the supernatants were pooled and kept at −20°C before measurement of IL-2, IL-4, IL-5, and IFN-γ using enzyme immunoassay kits (IEMA) from Coulter-Immunotech. If necessary, supernatants were diluted in PBS containing 3% (w/v) bovine serum albumin. Measurements from the pooled fraction were performed in duplicate. For the determination of cell proliferation, incubations were done in triplicate. Wells were pulsed with [³H]thymidine (0.2 µCi/well) for the last 18 h of culture. Then, cells were harvested (Tomtec Harvester 96; Dunns Laborotechnik, Asbach, Germany) and incorporation of [³H]thymidine into DNA was assessed using liquid scintillation. The IC₅₀ values were calculated after nonlinear regression analysis of the concentration-inhibition curve. The IC₅₀ values refer to the maximum inhibition achieved in the respective experiments, which was set to 100%.

Attenuation of Bradykinin-Induced Mucosal Leakage into the Rat Trachea by Topical Administration of Steroid

This experimental system has been originally described by Brattsand et al. (1991). For these experiments, steroids were dissolved in 0.9% NaCl containing 20% cyclodextrin (CD/NaCl) to give a stock solution of 300 µM. Further dilutions were done in CD/NaCl. Male Sprague-Dawley rats (300–400 g; Charles River Wiga) were anesthetized (60 mg/kg pentobarbitone), and the jugular vein and the femoral artery were cannulated. After exposure and partial dissection of the trachea, a segment of about 7 cartilage rings was superfused via polyethylene catheters with an infusion pump with 0.9% NaCl at 0.1 ml/min. Fractions of 1 ml were collected during every 10 min. A tube was secured into the descending trachea to allow spontaneous breathing.

Antiproliferative Activity and Involution of the Thymus and Adrenals in the Rat Cotton Pellet Granuloma Model

Experiments were done according to the procedure described previously (Winter et al., 1963). Two cotton pellets were implanted subcutaneously in the scapula region of male Sprague-Dawley CD rats (140–280 g; Charles River Wiga). One pellet was treated with a steroid solution, and the other was left untreated. Steroids were dissolved in absolute ethanol. Before implantation, 50 µl of drug solution was applied to the treated pellets, and the pellets were dried for 1 h. Inhibition of granuloma formation around the steroid-treated pellet indicates the local anti-inflammatory response; inhibition of granuloma formation around the nontreated pellet indicates the systemic anti-inflammatory response. Control animals received two nontreated pellets. Eight or 16 animals per dose were used. After 7 days, the animals were killed and the granulomas were excised and dried at 120°C for 15 h. The difference between the weight of the dried granulomas and the initial weight of the cotton pellets indicated the amount of granulation tissue per animal. The percentage decrease of the granuloma mean dry weight of the steroid-treated pellets compared with that of the control group was taken as a measure of anti-inflammatory activity. In addition the thymus and adrenals were removed and weighed. The ED₅₀ values were determined by nonlinear regression analysis.

Antigen-Induced Late Inflammatory Response in the Brown Norway Rat

Animals and Sensitization. Male BN rats (180–220 g at the beginning of sensitization) were sensitized by the following method. Briefly, on days 1, 14, and 21, the rats received a subcutaneous (neck injection) of 0.5 ml/rat saline solution containing 20 µg/ml OVA; grade V (Fluka, Buchs, Switzerland) adsorbed to 40 mg/ml Al(OH)₃; simultaneously, the animals were i.p. injected with 0.25 ml/rat Bordetella pertussis vaccine (Behringwerke AG, Marburg, Germany) diluted in saline containing 4 × 10⁶ heat-killed bacilli/ml. This method has been described in Bundschuh et al. (2001).

Experimental Protocol and Drug Administration. Steroids were mixed with lactose powder; the administered volume was 20 mg/kg for compounds and placebo. Ciclesonide and des-CIC were administered at doses of 0.1, 0.3, 1, 3, and 10 mg/kg and budesonide at doses of 0.1, 1, and 10 mg/kg. The drugs were administered i.t. 24 h and 1 h before OVA challenge. Nontreated OVA-challenged or nontreated nonchallenged controls received the equivalent amount of lactose 24 h and 1 h before the OVA challenge. OVA challenge was performed 28 days after the beginning of sensitization. Each animal was placed in a special tube to restrain its activity and to guarantee nose-only exposure. These tubes were connected to an inhalation tower (CR Equipment S.A., Tannay, Switzerland) that allowed simultaneous challenge of 32 animals. The OVA-containing aerosol was generated by filtered air at a pressure of 1.7 bar (600 l/h) using a medication nebulizer device (Hospitak; delivered by Carbamed, Basel, Switzerland) and a solution of 3.2 mg/ml OVA (grade V; Sigma Chemie; diluted in saline). The aerosol was moved forward by a continuous airflow of 2000 l/h. The exposure time was 1 h, resulting in an aerosolized OVA volume of about 20 ml. The nonchallenged controls (sham challenge) were sensitized with OVA and exposed to saline aerosol. Forty-eight hours after OVA/sham challenge, animals were anesthetized with Trapanal (thiopental, 150 mg/animal), and BAL was performed. The trachea of the rats was exposed and cannulated, followed by gently lavage of the lungs three times in situ with 4 ml per animal (~2 ml/100 g body weight) BAL buffer (Hanks' balanced salt solution containing 0.372% NaEDTA and 10 mM HEPES). On average, 80% of the administered BAL fluid was recovered regardless of pretreatment.

Cell Differentiation and Determination of Total Protein and TNF-α in Bronchoalveolar Lavage Fluid (BALF). Total cell counts and cell type differentials in BALF of placebo-treated/OVA-challenged and drug-treated/OVA-challenged rats were obtained im-
mediated after BAL using an automatic leukocyte differentiation system (Cobas Helios 5 Diff; F. Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany). For this purpose, BAL samples (about 3 ml) were directly applied to the analyzer and were automatically mixed with Minolysé (ABX, Göppingen, Germany), which lyses red blood cells, and with Eosinofix (ABX), which stabilizes leukocyte membranes and stains eosinophils. This procedure allows the two-dimensional differentiation of leukocytes by size (measured by a scatter light). For nonchallenged controls (sham challenge), total cell counts were determined using the cell counter Sysmex F-300 (Digitana AG, Hamburg, Germany). In parallel, leukocyte differentiation counts were determined using the cell counter Sysmex F-300 (Digitana AG, Hamburg, Germany). The respective ED50 and ED25 values were determined using linear regression.

Data Analysis. Biostatistical analysis of drug-induced individual changes (drug-treated versus solvent-treated OVA-challenged animals) was performed on the basis of medians using the Mann-Whitney U test. Monotone dose dependence was evaluated by the Jonckheere Terpstra test using all doses tested. p < 0.05 was considered statistically significant.

Adverse Effect Profile of Ciclesonide after Repeated Oral Administration for 28 Days

Corticosteroid stock solutions were prepared in ethanol as 20 mg/ml; further dilutions into vehicle (NaCl, 700 mg; sodium carboxymethylcellulose, 750 mg; Tween 80, 1 drop; aqua dest at 100 ml) were done at 1:500, 1:100, and 1:20. Of these solutions, 2.5 ml/kg body weight was applied via a gastric tube to male Wistar rats (obtained from the breeding facilities at Elmuchimica Farmaceutica S.L., Madrid, Spain), resulting in final doses of 100, 500, and 2500 μg/kg, respectively. Ten rats were used per dose. After 28 days, the decrease in gain of body weight and the decrease of the weight of thymus and adrenals were assessed. Control animals received vehicle only. The respective ED50 and ED25 values were determined using linear regression.

Results

Binding to the Glucocorticoid Receptor of Rat Lung.

The competitive binding assays were performed in the presence of the protease inhibitors PMSF or DFP. All binding data were referenced to the RBA of dexamethasone that was defined as 100. For each steroid, two independent binding experiments were performed, using duplicate measuring points (Table 1). The RBA of ciclesonide was about 8-fold lower than that of dexamethasone. In contrast, the RBA of des-CIC was 12-fold higher than the RBA of dexamethasone and 100-fold higher than that of ciclesonide, suggesting that des-CIC is the active metabolite of ciclesonide. The RBAs of the 2’R-epimers (parent compound or metabolite) were about 5- to 6-fold higher than the RBAs of the respective 2’S-epimers. The RBA of budesonide was 9-fold higher than the RBA of dexamethasone but slightly lower than the RBA of des-CIC. 16-OH-Prednisolone (a potential metabolite of des-CIC) had a low RBA, comparable with the one of the 2’S-epimer of ciclesonide (data not shown).

Inhibition of ConA-Induced Proliferation of Rat Spleen Cells.

All steroids inhibited the ConA-induced incorporation of [3H]thymidine into rat spleen cells in a dose-dependent manner. Three independent experiments were performed for all steroids, with the exception of BYK20432 (Table 2). Half-maximal inhibition (IC50) was obtained at 1.9 nM for ciclesonide, at 1.5 nM for des-CIC, and at 1.3 nM for budesonide. The 2’S-epimers BYK20427 and the respective metabolite BYK20433 were on average 7-fold less potent than the respective 2’R-epimers and inhibited half maximally at 13.8 and 10.0 nM, respectively. Des-CIC compared well with budesonide. In this assay, the potency of the parent compound ciclesonide was only slightly less than the potency of des-CIC, which is probably due to in vitro ester cleavage of ciclesonide at the C21-position.

Inhibition of ConA-induced proliferation of rat spleen cells by ciclesonide, its metabolite des-CIC and of the respective 2’S-epimers BYK20427 and BYK20433

<table>
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<tr>
<th>Steroid</th>
<th>IC50 (nM)</th>
<th>Mean ± S.D.</th>
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<tr>
<td>Ciclesonide</td>
<td>1.7 (1.2–1.6)</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Des-CIC</td>
<td>9.0 (7.5–10.7)</td>
<td>10.0</td>
</tr>
<tr>
<td>BYK20427</td>
<td>1.5 (1.3–1.6)</td>
<td>1.3 ± 0.2</td>
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<tr>
<td>BYK20433</td>
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<td></td>
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<tr>
<td>Budesonide</td>
<td>1.2 (0.9–1.5)</td>
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</table>

ND, not done.

**TABLE 2**

Inhibition of ConA-induced proliferation of rat spleen cells by ciclesonide, its metabolite des-CIC and of the respective 2’S-epimers BYK20427 and BYK20433

Spleen cells from Sprague-Dawley rats were stimulated with ConA in the presence or absence of steroid, concentration range: 10^-5 to 10^-11 M, eleven points per curve. Samples were set up in triplicate. After 72 h, a MTT assay was performed. Efficacy of inhibition ranged from 70 to 85%, depending on the rat. The IC50 values denote half-maximal inhibition and were determined using nonlinear regression; the 95% confidence interval is given in parentheses for the individual experiments.
Inhibition of CD3-Induced Release of IL-4 and IL-5 from Murine T42-lymphocytes (D10.G4.1). Asthma has been considered to be a T42-mediated disease, although recent publications challenge this view (Salvi et al., 2001). We therefore investigated the effect of ciclesonide and des-CIC on the release of the T42 cytokines IL-4 and IL-5 by cells from the murine T42-clone D10.G4.1 stimulated with a CD3 monoclonal antibody. Ciclesonide and des-CIC inhibited the release of IL-4 with an IC50 value of 0.42 and 0.19 nM and the release of IL-5 with an IC50 value of 0.27 and 0.12 nM, respectively (mean of two independent experiments). Budesonide inhibited the release of IL-4 and IL-5 with an IC50 value of 0.32 and 0.22 nM, respectively (Table 3).

Inhibition of ConA-Induced Proliferation of Human PBMCs. All glucocorticoids inhibited the ConA-induced incorporation of [3H]thymidine into human peripheral blood mononuclear cells in a concentration-dependent manner. Ciclesonide and des-CIC were tested in four independent experiments (Table 4). The IC50 value of des-CIC was comparable with the IC50 value of budesonide and, on average, was 7.7-fold lower than the IC50 value of ciclesonide. The 2'S-epimer BYK20427 was 4.7-fold less potent than ciclesonide. In a single experiment, BYK20433 (the metabolite of the 2'S-epimer BYK20427) inhibited the proliferation of ConA-activated PBMCs with an IC50 value of 6.79 nM (95% confidence limits, 5.0–9.2; data not shown).

Inhibition of CD3-Induced Proliferation and Cytokine Production by Purified Human CD4+ Lymphocytes. Highly purified CD4+ T-cells were stimulated with a combination of CD3 and CD28 mAb to mimic lymphocyte activation via the TCR/CD3 complex and the (probably) most important costimulatory molecule, CD28 (Lenschow et al., 1996). All compounds under investigation inhibited the respective read-out parameters proliferation and synthesis of IL-2, IL-4, IL-5, and IFN-γ in a concentration-dependent manner. Table 5 shows that ciclesonide was 6-fold more potent than the respective 2'S-epimer BYK20427 (except for the inhibition of IL-2). Generally, des-CIC was significantly more potent than the parent compound ciclesonide (except for the inhibition of IL-4), which supports the concept of ciclesonide being the parent compound of the active metabolite des-CIC. Des-CIC and budesonide showed comparable potency regarding the inhibition of cytokine synthesis; furthermore, in this system des-CIC was more potent than budesonide in inhibiting proliferation. BYK20433, which is the metabolite of the 2'S-epimer BYK20427, was 2.3- to 14-fold less potent than des-CIC, depending on the parameter investigated.

In vivo anti-inflammatory activity of ciclesonide was investigated in three animal systems: 1) the attenuation of bradykinin-induced leakage into the trachea of the rat after topical administration of the glucocorticoid; 2) the inhibition of allergen-induced accumulation of protein, eosinophils, and TNF-α in the BALF of OVA-challenged Brown Norway rats after inhalative administration of the glucocorticoid; and 3) the induction of anti-proliferative activity in the rat cotton pellet granuloma model.

**Attenuation of Bradykinin-Induced Mucosal Leakage into the Rat Trachea by Topical Administration of Steroid.** The spontaneous and bradykinin-induced release of plasma into the trachea was determined. Glucocorticoids were superfused for 10 min, and bradykinin was applied 70 min thereafter. The concentration-dependent inhibition of the bradykinin-induced leakage into the rat trachea by ciclesonide and budesonide with an ED50 value of 14.7 and 39.6 μM, respectively (Fig. 2), suggested a trend to higher potency of ciclesonide. Statistical significance was not achieved because the 95% confidence limits were overlapping.

**Accumulation of Eosinophils, TNF-α, and Total Protein in the Bronchoalveolar Lavage Fluid of Ovalbumin-Sensitized/Challenged Brown Norway Rats.** As shown in Fig. 3, ciclesonide, des-CIC, and budesonide inhibited in a dose-dependent manner the accumulation of eosinophils (A), total protein (B), and TNF-α (C) in the BALF. Ciclesonide, des-CIC, and budesonide inhibited the accumulation of eosinophils with an ED50 (95% confidence limits in parentheses) value of 0.5 mg/kg (0.30–0.89), 0.7 mg/kg (0.37–1.34), and 1.3 mg/kg (0.35–5.14); the accumulation of protein with an ED50 value of 0.6 mg/kg (0.39–0.87), 0.5 (0.34–0.82) mg/kg, and 0.7 mg/kg (0.29–1.71), respectively; and the accumulation of TNF-α with an ED50 value of 0.4 mg/kg (0.23–0.78), 0.4 mg/kg (0.23–0.65), and 0.9 mg/kg (0.19–4.18), respectively.

**Antiproliferative Activity and Involution of the Thymus and Adrenals in the Rat Cotton Pellet Granuloma Model.** Inhibition of granuloma formation at the steroid-treated pellet indicates the local anti-inflammatory action; the systemic anti-inflammatory action is detected at the non-treated pellet and via the reduction of the weight of the thymus and the adrenals. Table 6 shows the effect of ciclesonide, des-CIC, and budesonide on the development of cotton pellet granulomas in the rat. Ciclesonide showed high local antiproliferative activity (ED50 = 2 μg/pellet); des-CIC was about 20-fold less active than the parent compound, and budesonide was about 16-fold less active than ciclesonide. The 2'S-epimers BYK20427 and the respective metabolite BYK20433 were about 12- and 5.5-fold less potent than ciclesonide and des-CIC, respectively (data not shown). The development of granulomas around the untreated pellet was inhibited only at very high concentrations of the steroids. The inhibition at the maximum concentration used was 56% for ciclesonide and 46% for budesonide, whereas the 2'S-epimer BYK20427 did not inhibit at the untreated pellet.

A sensitive parameter for the detection of systemic steroid effects is the decrease of the weight of the thymus. Ciclesonide and des-CIC led to reduction in the weight of the thymus with an ED50 value of 303 and 279 μg/pellet, respec-
In general, six to eight concentration-inhibition curves were evaluated to calculate an average IC50. For inhibition of IL-4 synthesis, only three to four concentration-inhibition curves were evaluated as some cell preparations produced very low titers of IL-4.

Highly purified human CD4+ lymphocytes were stimulated with a combination of a CD3 mAb and a CD28 mAb in the presence or absence of seven concentrations of steroids ranging from 10−12 to 10−6 M. After 72 h, the incorporation of [3H]thymidine and the synthesis of cytokines were measured. Efficacy of inhibition was 80% for proliferation, and for synthesis of cytokines 90% (IL-2), 70% (IL-4), 90% (IL-5), 90% (IFN-γ). IC50 values denote half-maximal inhibition and were determined using nonlinear regression. In general, six to eight concentration-inhibition curves were evaluated to calculate an average IC50. For inhibition of IL-4 synthesis, only three to four concentration-inhibition curves were evaluated as some cell preparations produced very low titers of IL-4.

**TABLE 4**

Inhibition of ConA-induced proliferation of human PBMC by ciclesonide, its metabolite des-CIC, and by the respective 2'S-epimers BYK20427 and BYK20433

Human peripheral blood mononuclear cells were stimulated with ConA in the presence or absence of eleven concentrations of steroids ranging from 10−6 to 10−11 M. Samples were set up in triplicate. After 72 h, the incorporation of [3H]thymidine (expts. 2–4) or bromodeoxyuridine (expt. 1) was determined. Efficacy of inhibition ranged from 83 to 97%, depending on the donor. IC50 values denote half-maximal inhibition and were determined using nonlinear regression. The 95% confidence interval is given in parentheses for the individual experiments.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>IC50 (nM)</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expt. 1</td>
<td>Expt. 2</td>
</tr>
<tr>
<td>Budesonide</td>
<td>1.4 (0.8–2.2)</td>
<td>1.2 (1.0–1.5)</td>
</tr>
<tr>
<td>Ciclesonide</td>
<td>14.6 (9.1–23.4)</td>
<td>5.4 (4.1–7.1)</td>
</tr>
<tr>
<td>Des-CIC BYK20432</td>
<td>1.4 (0.9–2.1)</td>
<td>1.0 (0.7–1.3)</td>
</tr>
<tr>
<td>BYK20427</td>
<td>ND</td>
<td>30.8 (23.4–40.7)</td>
</tr>
</tbody>
</table>

ND, not done.

**TABLE 5**

Inhibition of CD3-induced proliferation and cytokine production in purified human CD4+ lymphocytes by ciclesonide, des-CIC, the respective 2'S-epimers BYK20427 and BYK20433, and by budesonide

Highly purified human CD4+ lymphocytes were stimulated with a combination of a CD3 mAb and a CD28 mAb in the presence or absence of seven concentrations of steroids ranging from 10−12 to 10−6 M. After 72 h, the incorporation of [3H]thymidine and the synthesis of cytokines were measured. Efficacy of inhibition was 80% for proliferation, and for synthesis of cytokines 90% (IL-2), 70% (IL-4), 90% (IL-5), 90% (IFN-γ). IC50 values denote half-maximal inhibition and were determined using nonlinear regression. In general, six to eight concentration-inhibition curves were evaluated to calculate an average IC50. For inhibition of IL-4 synthesis, only three to four concentration-inhibition curves were evaluated as some cell preparations produced very low titers of IL-4.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>IC50 (nM)</th>
<th>Proliferation</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-5</th>
<th>IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciclesonide</td>
<td>1.4 (0.6–2.9)</td>
<td>0.7 (0.5–1.0)</td>
<td>0.8 (0.3–2.0)</td>
<td>0.5 (0.3–0.8)</td>
<td>1.5 (0.9–2.4)</td>
<td></td>
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<tr>
<td>Des-CIC BYK20432</td>
<td>0.2 (0.1–0.5)</td>
<td>0.2 (0.1–0.3)</td>
<td>0.8 (0.2–2.4)</td>
<td>0.2 (0.1–0.3)</td>
<td>0.4 (0.2–0.6)</td>
<td></td>
</tr>
<tr>
<td>BYK20427</td>
<td>10.0 (5.8–10)</td>
<td>1.7 (0.7–4.1)</td>
<td>4.7 (1.4–16)</td>
<td>2.9 (1.5–6.0)</td>
<td>10.4 (5.0–15)</td>
<td></td>
</tr>
<tr>
<td>BYK20433</td>
<td>2.9 (1.5–5.8)</td>
<td>1.5 (0.9–2.5)</td>
<td>1.8 (0.9–4.0)</td>
<td>1.0 (0.6–1.7)</td>
<td>2.7 (1.7–4.3)</td>
<td></td>
</tr>
<tr>
<td>Budesonide</td>
<td>1.3 (0.8–2.1)</td>
<td>0.2 (0.07–0.7)</td>
<td>0.3 (0.2–0.5)</td>
<td>0.2 (0.1–0.3)</td>
<td>0.7 (0.5–1.1)</td>
<td></td>
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</tbody>
</table>

*Italic* numerals in brackets show the number of experiments.

Adverse Effect Profile of Ciclesonide after Repeated Oral Administration for 28 Days. The effect of the oral administration for 28 days of ciclesonide and budesonide to rats was investigated, using the involution of thymus and adrenals and the decrease in gain of body weight as indicators of systemic glucocorticoid action (Table 7). Ciclesonide was about 7-fold less active than budesonide regarding the involution of thymus, about 8-fold less active concerning the involution of the adrenals, and about 8-fold less active with regard to decreasing the gain of body weight. For a fair comparison, it should be kept in mind that in the rat the oral bioavailability of budesonide is 15% (Chanoine et al., 1991), whereas the oral bioavailability of ciclesonide is low; in the human system below 1% (Nave et al., 2002). Although the data suggest a trend for a better side effect profile of ciclesonide, the overlap of the 95% confidence intervals of the regression curves does not allow differentiation between the two compounds.

**Discussion**

We describe ciclesonide, a newly developed steroid, which is the 2'R-epimer of 2'-cyclohexyl-11β-hydroxy-21-isobutyryloxy-16H-dioxolo[5,4;16,17]pregna-1,4-diene-3,20-dione. Investigations regarding the binding of the compound to the glucocorticoid receptor of rat lung revealed that the metabolite des-CIC has a 100-fold higher affinity to the glucocorticoid receptor than the parent compound ciclesonide. A similar relationship between ciclesonide (K of 37 nM) and des-CIC (K of 0.51 nM) was seen with human glucocorticoid receptor from Jurkat cells (Belvisi et al., 2002). Des-CIC has been described as the major metabolite of ciclesonide in the rat and in human (Hall et al., 2000). These findings support the concept that ciclesonide is the parent compound of des-CIC. Furthermore, the binding data indicate that the 2'S-epimer BYK20427 and its metabolite BYK20433 have a lower affinity for the glucocorticoid receptor than the respective 2'R-epimers. This observation led to the development of the 2'R-epimer instead of the S-epimer. Comparison with budesonide revealed a slightly higher affinity of des-CIC for the rat glucocorticoid receptor. Comparison with literature data in the same system suggests that the RBA of des-CIC (RBA = 1212) is comparable with that of beclomethasone monopropionate (RBA = 1345) and is higher than that of triamcinolone acetonide (RBA = 361) or betamethasone (RBA = 55) (Wuerthwein et al., 1992).

The T-cell mitogen ConA induces proliferation of T-lymphocytes, which can be suppressed by glucocorticoids (Smith et al., 1977; Gillis et al., 1979). Ciclesonide and des-CIC inhibited the ConA-stimulated proliferation of rat spleen cells in a concentration-dependent manner, whereby ciclesonide and des-CIC had the same potency. This result suggests that in these cells, ciclesonide can easily be converted into des-CIC. Budesonide has the same potency as des-CIC. It is noteworthy that the 2'S-epimer BYK20427 and
leakage into the trachea was induced by 10^(-6) M bradykinin in either steroid-treated or untreated rats. Steroid treated groups involved seven to eight animals, solvent-treated control groups involved 15 animals. ND, not done. The 95% confidence intervals of the IC_{25} values were overlapping.

its respective metabolite BYK433 were about 7-fold less potent than the corresponding 2'R-epimers, and this is in agreement with the results of the glucocorticoid receptor binding affinities. In the murine T_{H}2-clone D10.G4.1, ciclesonide and des-CIC inhibited the synthesis of IL-4 and IL-5. Both cytokines are thought to be key mediators in chronic allergic inflammation, by inducing the switch to IgE and in supporting the survival of eosinophils (Lord and Lamb, 1996).

Human PBMCs are a mixture of lymphocytes and monocytes; stimulation with ConA induces monocyte-dependent proliferation in T-lymphocytes. With regard to the inhibition of the ConA-stimulated proliferation of human PBMCs, des-CIC and budesonide were equally potent, whereas ciclesonide was less potent. Highly purified human CD4^+ lymphocytes that are essentially devoid of monocytes can be activated by a combination of a matrix-bound mAb directed against the CD3/TCR complex and a so-called “second stimulus”, the prototype of which is stimulation via CD28. In the latter system, des-CIC was more potent than budesonide in inhibiting CD3/CD28-induced proliferation. The lower potency of budesonide cannot be explained because both compounds inhibited the production of IL-2 at comparable concentrations; the inhibition of IL-4, IL-5, and IFN-γ production was also comparable. In both systems, the parent compound ciclesonide was less active than des-CIC, supporting the concept that ciclesonide acts as a parent compound and that the conversion of ciclesonide to des-CIC was incomplete in this system. Comparing the potency of ciclesonide and des-CIC in human and murine lymphocytes, it can be seen that the difference in potency between both compounds was more pronounced in human cells. Although the esterases involved in the activation of ciclesonide are not yet characterized, a recent abstract (Mutch et al., 2003) suggests a role for the rather ubiquitously expressed carboxylesterases. In our experiments, murine sera were relatively potent in activating ciclesonide. Furthermore, when comparing the potency of ciclesonide and des-CIC in inhibiting the CD/CD28-induced proliferation or cytokine release from purified human lymphocytes (Table 5) and the release of TNF-α from LPS-stimulated dendritic cells or monocytes (Stoeck et al., 2001), we observed a maximally 7-fold difference in potency between ciclesonide and des-CIC in human lymphocytes and a 30-fold difference in human dendritic cells and monocytes. Whether this is due to an equipment of these cells with different esterases is unclear at present.

Overall, the in vitro results indicate that des-CIC has a potent anti-inflammatory effect, which is comparable with that of budesonide. Depending on the experimental system used, ciclesonide can be transformed into its active metabolite in vitro.

In vivo, ciclesonide proved to be potent in three experimental systems: 1) attenuation of bradykinin-induced mucosal leakage into the rat trachea after topical administration of the glucocorticoid; 2) inhibition of allergen-induced accumulation of protein, eosinophils, and TNF-α in the BALF of OVA-challenged Brown Norway rats after inhalative administration; and 3) induction of antiproliferative activity in the rat cotton pellet granuloma model.

Bradykinin can change vascular permeability and increase extravasation of plasma proteins. Inhibition of bradykinin-induced mucosal plasma extravasation is a known but unspecific effect of glucocorticoids. Even when ciclesonide was administered for only 10 min via the superfusion fluid 70 min before the bradykinin provocation, ciclesonide had a concentration-dependent effect on the tracheal mucosal plasma leakage, showing a trend for increased potency compared with budesonide. These findings indicate, that ciclesonide enter rapidly into the tracheal mucosa and initiated its activity or was not completely washed out by the subsequent 70-min superfusion. Others have described similar effects for the inhibition of platelet-activating factor-induced mucosal leakage by dexamethasone (Boschetto et al., 1991).

The action of ciclesonide in the system described above represents a fast-acting anti-inflammatory effect. In contrast, the inhibition of the allergen-induced accumulation of eosinophils and protein as well the release of TNF-α, as detected in the model of the OVA-sensitized and -challenged BN rat, is more protracted. Infiltration of inflammatory cells and edema formation are common features of human asthma and are also observed in OVA-sensitized and -challenged BN rats (Elwood et al., 1991; Schneider et al., 1997). Ciclesonide and des-CIC potently inhibited the experimental parameters under investigation; budesonide showed comparable effects.

The local anti-inflammatory effect of ciclesonide was investigated also in the rat cotton pellet model that is widely used for characterizing of anti-inflammatory drugs (Winter et al., 1963; Loux et al., 1977). The inflammation in this model is long-lasting. The model has the additional advantage that the local anti-inflammatory effect, detected at the steroid-
treated pellet, can be compared with the systemic effects, i.e., the inhibition of granuloma formation around the nontreated pellet and the involution of the thymus and adrenals. The reduction of the weight of the thymus has been described as a very sensitive parameter for the detection of systemic glucocorticoid action (Ben Rhouma and Sakly, 1994). Very low doses (ED\textsubscript{50}/H\textsubscript{11005} = 2 mg/pellet) of ciclesonide inhibited the formation of the granuloma around the steroid-treated pellet; the ED\textsubscript{50} for the reduction of the thymus weight was 150-fold higher. In contrast, the antiproliferative activity of des-CIC at the treated pellet was about 20 times lower than that of treated pellets.

### TABLE 6

<table>
<thead>
<tr>
<th>Steroid</th>
<th>ED\textsubscript{50} (µg/pellet)</th>
<th>Local Antiproliferative Activity</th>
<th>Involution of Thymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciclesonide BYK20426</td>
<td>2 (1–3)\textsuperscript{a}</td>
<td>303 (195–470)</td>
<td>303 (195–470)</td>
</tr>
<tr>
<td>Budesonide</td>
<td>33 (24–47)</td>
<td>154 (115–205)</td>
<td>154 (115–205)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} 95% confidence interval.

### TABLE 7

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Involution of Thymus</th>
<th>Involution of Adrenals</th>
<th>Decrease in Gain of Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciclesonide BYK20426</td>
<td>2226</td>
<td>1746</td>
<td>2166</td>
</tr>
<tr>
<td>Budesonide</td>
<td>339</td>
<td>214</td>
<td>261</td>
</tr>
</tbody>
</table>

### Fig. 3

Inhibition of antigen-induced eosinophil (A) influx, total protein (B), and TNF-α (C) accumulation in BALF of OVA-challenged BN rats by intratracheally administered ciclesonide (□), des-CIC (△), and budesonide (▼). Data are shown as mean ± S.E.M.; n = 8 to 16 animals per steroid dose, n = 12 per placebo group. The 95% confidence intervals of the ED\textsubscript{50} values are given under Results.

**In summary,** the in vivo data shown here underline the strong local anti-inflammatory potency of ciclesonide and the proposed reduced side effects compared with the topical administration of des-CIC or budesonide. This strong local action is due to several properties of ciclesonide: 1) local ester-


References