PRE-CLINICAL PROFILE OF CICLESONIDE, A NOVEL
CORTICOSTEROID FOR THE TREATMENT OF ASTHMA.

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Running title: Ciclesonide, a novel corticosteroid.

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Non standard abbreviations: des-CIC, desisobutyryl-ciclesonide; GR, glucocorticoid receptor; PR, progesterone receptor; TR, testosterone receptor; BAL, Bronchoalveolar lavage; GRE, glucocorticoid response element;

Section assignment: Inflammation and immunopharmacology
ABSTRACT

Ciclesonide is a novel, inhaled corticosteroid under development for the treatment of asthma. Ciclesonide is activated to desisobutyryl-ciclesonide (des-CIC) in the lungs to provide potent anti-inflammatory activity. The investigations herein compared the activity of ciclesonide with fluticasone in animal models to assess efficacy/potency as an airway anti-inflammatory and the comparative side effect potential in order to consider the therapeutic ratio of each compound. In radioligand binding assays, des-CIC and fluticasone exhibited comparable high affinity binding to the glucocorticoid receptor while ciclesonide exhibited 100-fold less binding affinity. In the Brown Norway rat model of antigen-induced airway eosinophilia, and in a model of sephadex-induced lung oedema, ciclesonide and fluticasone exhibited comparable efficacy. Interestingly, following 7-day intratracheal administration, ciclesonide elicited adrenal involution with a potency that was 44-fold less than fluticasone. Furthermore, ciclesonide was 22-fold less active than fluticasone in eliciting hypoplasia of the femoral growth plate. These data support the concept that ciclesonide acts as a parent compound that, when delivered to the airways, can be transformed into the active metabolite, des-CIC, resulting in local high anti-inflammatory activity. Furthermore, ciclesonide possesses equivalent anti-inflammatory efficacy through pulmonary activation with a significantly improved safety profile in pre-clinical animal models compared to fluticasone.
INTRODUCTION

Inhaled corticosteroids are the most effective prophylactic therapy currently available for the treatment of asthma, particularly in patients with mild to moderate asthma and persistent symptoms (Barnes, 1998). Airway inflammation is thought to underlie the increased airway responsiveness seen in asthma (Barnes, 1996) and inhaled corticosteroids reduce airway responsiveness to a variety of direct and indirect stimuli in patients with mild asthma (Henriksen and Dahl, 1983; Vathenen et al., 1991; O’Connor et al., 1992). Steroids are thought to possess this beneficial therapeutic profile principally via their anti-inflammatory properties and regular treatment has been shown to improve lung function, control exacerbations and attenuate surrogate markers of airway inflammation such as a the percentage of eosinophils in induced sputum (Jatakanon et al., 2000).

Although the currently available steroids (including budesonide, fluticasone propionate and mometasone furoate) have a reasonable therapeutic ratio, due to the removal of the swallowed fraction of the drug by the hepatic metabolism, these drugs can be directly absorbed from the lung mucosa, and therefore systemic effects can often be observed at the higher doses (Barnes, 1998). In fact, the systemic side-effects associated with the long-term use of inhaled corticosteroids are still a cause for concern. These side effects include suppression of the hypothalamic-pituitary (HPA) axis, osteoporosis, and reduced bone growth in the young, opportunistic infections, behavioural alterations, disorders of lipid metabolism, oral candidiasis, and glaucoma (Schäcke et al, 2002). Therefore, a major challenge for the pharmaceutical industry is the development of “safer” steroids with an improved therapeutic window (Belvisi et al., 2001a).
Ciclesonide is a new inhaled corticosteroid currently under clinical development for the treatment of asthma. It is a non-halogenated ester parent compound that is converted in the lung by esterases to form the active metabolite desisobutyryl-ciclesonide (des-CIC); (Dietzel et al., 2001; Dent, 2002). Ciclesonide has extremely low oral bioavailability (Nave et al., 2002) and is highly sensitive to metabolism by liver oxidases. The first clinical data on ciclesonide have already been published and have demonstrated that ciclesonide reduces airway responsiveness to adenosine-5'-monophosphate in a dose-dependent manner when compared to placebo in patients with mild to moderate asthma. A reduction in eosinophils was also observed in induced sputum from the same patients (Taylor et al., 1999). Another study has shown that ciclesonide produces a significant inhibition of early and late phase reaction after allergen challenge (Dahl et al., 1998). Furthermore, a more recent clinical study has shown that ciclesonide given once daily is effective in the treatment of mild-to-moderate asthma as assessed by lung function, symptoms, use of rescue medication and number of asthma exacerbations (Postma et al., 2001).

In the current study we have established the pre-clinical pharmacological profile of the novel corticosteroid, ciclesonide and compared it to the standard comparator fluticasone propionate. These studies were performed with a view to establishing the efficacy of ciclesonide as an anti-inflammatory agent in standard animal models of eosinophilic inflammation compared to fluticasone. Furthermore, animal models were configured to examine the systemic side effect profile of ciclesonide compared to fluticasone. In particular effects on adrenal involution were measured and decrease in the femur growth plate width, were used as a measure of steroid-induced osteopenia.
METHODS

Receptor binding studies

Radioligand binding experiments to evaluate the affinity of ciclesonide, des-CIC and fluticasone for the human glucocorticoid (GR), progesterone (PR) and estrogen receptors, and the rat testosterone receptors (TR) were performed as previously described (Schlecte et al., 1982; Eckert and Katzenellenbogen, 1982; Schilling and Liao, 1984; Steiner and Wittliff et al., 1985; Sheen et al., 1985). In each experiment, the respective reference compound was tested simultaneously at a minimum of eight concentrations in duplicate to obtain a competition curve in order to validate the experiment.

Assessment of serum protein binding

Serum protein binding of $^{14}$C-ciclesonide (10, 50, 100, 1000, 10000 ng/ml) and des-CIC (0.5, 5.0, 25, 100 and 500 ng/ml) was determined in Sprague-Dawley rats. Free and bound drug was separated by ultrafiltration (Centrifree Micropartition device) and quantified by scintillation counting.

Brown Norway rat model of antigen-induced airway inflammation

For all in vivo experiments, compounds were prepared as suspensions by grinding and sonicating the solid in 0.5% methyl cellulose / 0.2% Tween 80 in saline. UK Home Office guidelines for animal welfare based on the Animals (Scientific Procedures) Act 1986 were strictly observed.
Rats (male 250-300g, Brown Norway) were sensitised on experiment days 0, 12 and 21 with ovalbumin (100 µg, i.p.) administered with aluminium hydroxide adjuvant (100 mg, i.p.). Each day, between days 27 and 30, rats were challenged (once per animal) with inhaled antigen aerosol (10 g/l, 30 minutes). 24 h and 1 h before antigen challenge, rats were anaesthetised with halothane (4 % in O₂, 3-5 minutes) 24 h and 1 h before antigen challenge to allow intratracheal instillation of a test compound or vehicle. Rats were sacrificed (sodium pentobarbitone, 200 mg/kg, i.p.) 24 hours after antigen challenge followed by cannulation of the trachea. Cell influx into the airway lumen and lung tissue was quantified as previously described (Underwood et al., 1997, 2002).

**Sephadex-induced lung oedema in the Sprague-Dawley rat.**

Sephadex was made up in sterile saline (10 mg/ml) and allowed to swell for at least three days at room temperature. The test compounds were administered intratracheally 24 hrs before Sephadex administration, and concomitantly with intratracheal administration of Sephadex (5 mg/kg) under halothane anaesthesia (4% in oxygen for 3mins). The rats were sacrificed with pentobarbitone sodium (200 mg/kg, i.p.) 24 hours after Sephadex administration; body weight was recorded followed by the removal of the heart and lungs *en bloc*. Next, the lungs were excised and determination of wet lung weights along with correction for 100g initial body weight performed as previously described (Belvisi et al., 2000; Haddad et al., 2002).

**Osteopaenia of the femoral growth plate in the Sprague-Dawley rat**

The glucocorticoids were administered daily by the intra-tracheal route for 7 days under halothane anaesthesia (4% in oxygen for 3 to 4 mins). The adrenal glands and thymic glands

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were then removed and wet weights determined and corrected for 100g initial body weight. The
dose causing a 25% reduction (ED\textsubscript{25}) in adrenal weight and 50% reduction in thymic weight
(ED\textsubscript{50}) was determined. The left femur was removed with head intact in the acetabulum by
cutting through the pelvic girdle and through the femur shaft above the knee joint as previously
described (Belvisi et al., 2001b). The effective dose causing a 20% inhibition of growth plate
width (ED\textsubscript{20}) compared to the vehicle control group was determined for each compound.

Materials

Estrogen receptor, PR and TR receptor binding assays (study reference number: 992014) were
performed by CEREP (l’Evescault, France). The GR receptor binding assay was performed by
Panlabs (Taipei, Tiwan). Fluticasone propionate was synthesised by the Chemistry Department,
Aventis Pharma (Dagenham Research Centre, Essex, UK). Ciclesonide and des-CIC were
synthesised by Altana Pharma (Konstanz, Germany) and all other materials were purchased from
Sigma (Poole, U.K) except for: aluminium hydroxide from Prolabo (Fontenay, France), sodium
pentobarbitone (Euthatal) and halothane from Rhône Merieux (Harlow, UK). RPMI 1640 and
foetal calf serum was obtained from Gibco (Paisley, Strathclyde, Scotland, UK). Sephadex G-
200 was purchased from Pharmacia (Uppsala, Sweden). Carboxymethylcellulose (sodium salt),
paramat extra paraffin wax, xylene, acid fuchsin, and Alcian blue 8GX were supplied by BDH
Merck (Lutterworth, UK). Neutral buffered Formalin (10%) was supplied by Surgipath Europe
(St Neots, UK). Citric acid and formic acid were supplied by Aldrich Chemical (Gillingham,
UK). Saline (0.9% w/v) was obtained from Fresenius (Basingstoke, UK). Haematoxylin and
eosin Y were prepared stains from Shandon (Runcorn, UK).
Results

Receptor binding studies

des-CIC and fluticasone fully inhibited [\(^3\)H]dexamethasone binding with a 20- to 30-fold higher binding affinity for the human GR than dexamethasone. The active metabolite des-CIC was about 100-fold more potent than the parent compound ciclesonide (Table 1; Figure 1). des-CIC exhibited a similar binding affinity for the human GR receptor compared to fluticasone, suggesting that des-CIC is an equally potent anti-inflammatory agent as fluticasone. As expected, ciclesonide, des-CIC and fluticasone did not appreciably bind to the estrogen receptor, PR or TR (data not shown).

Protein binding data

The in vitro protein binding results of ciclesonide and des-CIC when expressed as the mean percentage of drug bound to serum and plasma proteins, respectively was high. The extent of protein binding in the rat was 98.6 - 98.9% (for ciclesonide) and 96.86 - 97.65 % (for des-CIC). No apparent saturation of protein binding was observed in the concentration range of 10 to 10000 ng/ml of ciclesonide and 0.5 to 500 ng/ml of des-CIC.

Brown Norway rat model of antigen-induced airway inflammation

Bronchoalveolar lavage (BAL) revealed a significant influx of eosinophils into the airway lumen after antigen challenge (Figure 2A). Eosinophilia was suppressed by both ciclesonide and fluticasone in a dose related manner. The ED\(_{50}\)'s for ciclesonide and fluticasone were 0.75 and
0.095 mg/kg, (i.t.), respectively. Antigen challenge also resulted in a significant accumulation of eosinophils in the lung tissue (Figures 2B). Both ciclesonide and fluticasone significantly inhibited this accumulation in a dose related fashion. The ED$_{50}$’s for ciclesonide and fluticasone were 0.49 and 0.068 mg/kg, (i.t.), respectively, and were within 10-fold from each other. The efficacy of both ciclesonide and fluticasone at inhibiting BAL and lung tissue eosinophilia was complete.

**Sephadex-induced lung oedema in the Sprague-Dawley rat.**

Sephadex instillation evoked a significant oedema response in the lung, which represented a 29.6% increase in wet lung weight, p< 0.01. Both ciclesonide and fluticasone significantly inhibited this increase in wet lung weight in a dose dependent manner. The ED$_{50}$ for ciclesonide and fluticasone were within 10-fold of each other (0.72 mg/kg and 0.08 mg/kg, i.t., respectively) (see Figure 3).

**Seven-day treatment side effect model in the Sprague-Dawley rat**

Both ciclesonide and fluticasone produced a dose-dependent decline in body weight gain. The dose of ciclesonide (0.01 – 10mg/kg/day) that inhibited the gain by 50% (ED$_{50}$) was 0.2 mg/kg/day. In contrast, the ED$_{50}$ for fluticasone (0.01 – 1mg/kg/day) was 0.02 mg/kg/day. Rats receiving 10mg/kg/day of fluticasone were culled prior to end point assessment due to excessive weight loss (> 20% initial body weight).
**Adrenal and thymic involution**

Decreases in adrenal weight were both dose dependent and significant for both compounds (see Figure 4). Fluticasone produced an apparent reduction at doses as low as 0.01 mg/kg/day. The dose of fluticasone causing a 25% reduction in adrenal weight (ED$_{25}$'s) was 0.070 mg/kg/day (i.t.). In contrast, ciclesonide produced no adrenal involution until doses above 0.10 mg/kg/day. The ED$_{25}$ was 3.11 mg/kg/day (i.t.). Both ciclesonide and fluticasone produced decreases in thymic weight. The dose of ciclesonide that decreased thymic weight by 50% (ED$_{50}$) was 0.32 mg/kg/day. In contrast, the ED$_{50}$ for fluticasone was 0.05mg/kg/day. Thus, compared to fluticasone, ciclesonide was 44.4 fold less potent at inducing adrenal involution and 6.4-fold less potent at inducing thymic involution.

**Femoral Head Histology**

Femur growth plate width changes from control with steroid treatment are shown in Figures 5 and 6. A dose related decrease in growth plate width was observed with fluticasone treatment resulting in a significant reduction compared to control at all doses (p<0.01). Maximal response was at 0.1 and 1.0 mg/kg/day (29% and 31%, inhibition respectively, with ED$_{20}$ 0.04 mg/kg/day). Ciclesonide treatment, also produced a dose dependent decrease in growth plate width. Effects began at a dose of 0.1mg/kg/day (p<0.01). Maximal inhibition was 23% at 10 mg/kg/day (ED$_{20}$ 0.87 mg/kg). Some reduction of osteopaenic effect was indicated at the 10mg/kg/day dose (21%).Thus, Ciclesonide was 22 fold less potent than Fluticasone at decreasing bone growth.
Morphology of the growth plate following administration of fluticasone 0.1 and 1mg/kg/day showed significant resorption within the metaphyses (osteoclast action bordering the hypertrophic zone) and cellular hypoplasia of the zone of proliferation (Figure 6). Ciclesonide, which produced a significant reduction of growth plate width, did not appear to present with hypoplasia of the proliferative zone, although an increased resorption within the metaphyses was observed.
DISCUSSION

Glucocorticoids are the mainstay of asthma treatment; however, in many cases, major side effects limit their therapeutic usefulness (Barnes, 1998). Side effects occur most commonly during long-term oral administration of glucocorticoids, due to high systemic exposure. However, even topical therapy can induce not only local (e.g. oral candidiasis), but also systemic side effects (e.g. osteoporosis, HPA axis suppression, growth retardation, cataract formation, skin bruising and thinning) (Cave et al., 1999; Lipworth, 1999; Lane, 2001).

The effects of the modern, synthetic glucocorticoids are mediated by the glucocorticoid receptor (GR). In brief, upon glucocorticoid binding the receptor, it translocates to the nucleus, where it can bind to a glucocorticoid response element (GRE) in a target gene promoter and initiate gene transcription (a process known as transactivation) (Beato et al., 1989). Negative regulation occurs when activated GR binds to negative GREs, leading to the discontinuation of gene transcription. Alternatively, the GR, activated by ligand, may interact with other transcription factors preventing an activation of transcription without direct DNA binding (processes know as transrepression) (Heck et al., 1994; Barnes, 1998 Barnes, 2001). Based on the large number of genes regulated in this way, including pro-inflammatory genes, a hypothesis was formulated that the transrepression mechanism was largely responsible for GR-mediated anti-inflammatory reactions and that transactivation mediated side effects (Reichardt et al., 1998; 2001; Schäcke et al., 2002). Evidence to support this hypothesis has been provided by a study investigating the glucocorticoid responsiveness in GR mutated mice (GR^{dim/dim}). A mutation in the GR of these mice prevents dimerisation of the receptor and, thereby, the capacity for DNA binding. Interestingly, all DNA-dependent regulatory mechanisms of the GR are disrupted but
the classical glucocorticoid-mediated anti-inflammatory effects were observed in GR\textsuperscript{dim/dim} mice (Reichardt et al., 1998; 2001). In contrast, for several of the side effects, it has been demonstrated that some effects are mediated predominantly via transrepression (eg. skin atrophy, suppression of HPA axis), whereas others are mediated predominantly by transactivation (diabetes mellitus, glaucoma) and some (eg. osteoporosis) are thought to be mediated by both (Schäcke et al., 2002). Currently used glucocorticoids cannot discriminate between the two processes.

One approach to synthesise efficacious but safe steroids has focused on physicochemical properties of inhaled corticosteroids that facilitate optimal pharmacological effects while minimising unwanted side effects. Ciclesonide was developed using this approach. It is a novel inhaled non-halogenated glucocorticoid that is activated in the lungs, through cleavage of C21-ester bond to form an active metabolite, desisobutyryl ciclesonide (\textit{des}-CIC), from the parent compound. \textit{des}-CIC, which has high local anti-inflammatory activity in the lung, is conjugated to fatty acids to prolong anti-inflammatory activity in the lung, is essentially devoid of oral bioavailability, has high protein binding in the systemic circulation and is rapidly and completely eliminated from the body (Dietzel et al., 2001; Dent, 2002). Recent data suggests that the oropharyngeal deposition of ciclesonide is only half that of fluticasone, with little activation to \textit{des}-CIC, following inhalation from a hydrofluoroalkane-propelled metered-dose inhaler in asthmatics suggesting a decreased likelihood of ciclesonide-associated side effects (Richter et al., 2005).

We found that \textit{des}-CIC and fluticasone exhibited high GR binding affinity with nanomolar concentrations. Ciclesonide was 100-fold less potent than \textit{des}-CIC in its ability to bind GR, thus confirming the hypothesis that ciclesonide is the parent compound and \textit{des}-CIC is
the active metabolite. Neither des-CIC nor fluticasone bound to the ER, PR or TR to any appreciable extent, indicating high specificity for the GR. In contrast, fluticasone has been shown to be a weak agonist at the PR when evaluated in cell based functional systems (Austin et al., 2002). The difference between the two studies may be due to the assays employed ie. a simple binding assay compared to a functional system that measures downstream events following receptor binding and activation. The similar binding affinities of des-CIC and fluticasone, at least for the human GR, suggest that the anti-inflammatory potency and efficacy of the two compounds may be similar. However, the relative potency of corticosteroids is best assessed in functional in vitro assays of anti-inflammatory activity. In studies of this sort the active moiety of ciclesonide and budesonide were roughly equipotent at repressing the activity of the pro-inflammatory transcription factor nuclear factor -κB in A549 lung epithelial cells, while fluticasone was approximately 10-fold more potent (Biggadike et al., 2004). However, in vitro potency alone does not predict in vivo activity as pharmacokinetic profile and drug delivery devices influence both pulmonary efficacy and therapeutic ratio.

In this study, ciclesonide completely suppressed antigen-induced lung and BAL eosinophilia in the Brown Norway rat model and sephadex-induced lung oedema. In both models, the efficacy of ciclesonide was identical to that of fluticasone and the ED₅₀ for ciclesonide was less than 10-fold higher than that of fluticasone. This data is consistent with a recent paper describing the activity of ciclesonide in a similar Brown Norway rat model of allergic inflammation (ED₅₀ = 0.7 mg/kg for inhibition of BAL eosinophilia compared to 0.75 mg/kg in this study) (Stoeck et al., 2004). These investigations used an acute treatment with ciclesonide, whereas inhaled corticosteroids are administered on a chronic basis for optimal anti-inflammatory therapy in clinical practice. Therefore, such small differences in potency between
Ciclesonide and fluticasone observed here may not be clinically meaningful when ciclesonide is administered daily for effective treatment of persistent asthma. Furthermore, given the lipophilic nature of ciclesonide and des-CIC (Nave et al., 2004) as well as the ability of des-CIC to form lipid conjugates within the lung – properties that prolong residency time and local anti-inflammatory activity – any minor differences in potency between ciclesonide and fluticasone may be effectively negated with chronic therapy. These points are supported by recent investigations in which daily administration for 21 days with ciclesonide inhibited both bronchial hyperresponsiveness and airway inflammation in an allergic asthma model, while fluticasone inhibited airway inflammation but not bronchial hyperresponsiveness (Leung et al., 2003). Furthermore, fluticasone caused systemic side effects at higher doses, while there was no effect on body weight or the HPA axis with ciclesonide. This data is consistent with clinical data showing that ciclesonide, dosed for 7 days at a clinically relevant dose, does not significantly impact on the HPA axis (Weinbrenner et al., 2002). It would have been interesting, as part of this study, to investigate the effect of ciclesonide on airway hyperreactivity (AHR). However, we have previously published that AHR is not evident in this particular Brown Norway allergic rat model (Underwood et al., 2002).

A major aim of this study was to assess the side effect potential of fluticasone and ciclesonide. Inhibition of bone growth is one of the major limitations of steroid usage. The growth plate region of mammalian long bones contains glucocorticoid sensitive elements within the proliferative zone. Activation of these elements by exogenous steroids leads to hypoplasia of this zone and reduced cell cycling in the growth plate. The long term physiological consequence of this is a reduction in long bone growth rate. Previous studies in this laboratory have demonstrated that morphometry of the femoral growth plate is a sensitive marker of steroid
impact upon bone (Belvisi et al., 2001b). In these studies we assessed steroid-induced osteopaenia of the femoral growth plate and adrenal involution in the Sprague-Dawley rat as indicators of steroid sensitive biomarkers indicative of side effect potential. Ciclesonide was found to be 44 fold and 22-fold less potent than fluticasone at eliciting adrenal involution and hypoplasia of the femoral growth plate, respectively, suggesting that ciclesonide may cause significantly less adrenal suppression than fluticasone. The differences between ciclesonide and fluticasone were apparent but less pronounced when body weight change and thymic involution were considered. The reasons for the discrepancy amongst these four systemic side effects are unclear but could possibly be due to the varying sensitivities of the tissues to the effects of steroids. The reasons for the reduced side effect profile of ciclesonide compared to fluticasone is not clear but may, at least in part, be due to the extremely high degree of protein binding observed with both ciclesonide and des-CIC (approx 97-99%) compared to fluticasone (approx 90%) (Derendorf et al., 1998). These data together with the demonstrated rapid clearance of ciclesonide from the systemic circulation could help to explain the superior therapeutic index described in this paper for ciclesonide compared to fluticasone.

In conclusion, this study demonstrates that ciclesonide is a novel inhaled corticosteroid that possesses a combination of physicochemical properties that enables equivalent anti-inflammatory efficacy but a significantly improved safety profile in pre-clinical animal models compared to fluticasone. The similar binding affinities observed in vitro may explain the similarities between the two compounds in anti-inflammatory potency/efficacy but cannot explain differences in the side effect profile. Other physicochemical characteristics such as on site-activation in the lung, lipid conjugation in the lung, high protein binding and extensive clearance and elimination are more likely to explain the differences. These data support the
proposition that ciclesonide has an improved therapeutic ratio in pre-clinical studies. Ciclesonide may become an important addition to the armamentarium of anti-inflammatory agents for the treatment of asthma.
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Figure Legends

Figure 1

Binding characteristics of ciclesonide, des-CIC and fluticasone for the human glucocorticoid receptor. The compounds were tested, in the presence of the protease/elastase inhibitor PMSF (0.6 mM), at 10 concentrations ranging from 0.3 nM to 10 µM in duplicate to obtain competition curves against radiolabelled dexamethasone. The specific radioligand binding to the receptors is defined as the difference between total binding and non-specific binding determined in the presence of an excess of unlabelled ligand. Results are expressed as a percent of control specific binding and as a percent inhibition of control specific binding obtained in the presence of the test compounds. IC₅₀ values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (nH) were determined by non-linear regression analysis of the competition curves. The equilibrium inhibition constants (Ki) were calculated according to the Cheng and Prusoff equation (Ki = IC₅₀/(1+L/Kₐ)), where L = concentration of radioligand in the assay, and Kₐ = affinity of the radioligand for its receptor).

Figure 2

The effect of ciclesonide and fluticasone on the antigen-induced influx of eosinophils into the airway lumen (A) and lung tissue (B) of ovalbumin sensitised and challenged Brown Norway rats. Compounds were administered by intratracheal administration into the airways 24 h and 1h before challenge by exposure to inhaled antigen. Cell numbers in BAL fluid and lung tissue are expressed as cells / ml and cells / mg respectively. Results are presented as mean ± s.e.mean of n = 12 observations. The statistical significance of differences between group data was determined...
using the Kruskal-Wallis multiple comparison test followed by a Dunnett’s post test with $P < 0.05$ accepted as significant ($* = P < 0.05$). For each compound an ED$_{50}$ value was calculated as the doses causing 50% inhibition of cell accumulation (where cell accumulation was defined as the increase in cell number between the unchallenged and the challenged, vehicle-treated control groups).

**Figure 3**
Effect of ciclesonide and fluticasone on Sephadex-induced changes in wet lung weight in conscious, Sprague-Dawley rats. Compounds were administered by intratracheal administration into the airways 24 h before and together with the Sephadex administration. Data are expressed as percentage inhibition of lung weight and presented as mean ± s.e. mean of n = 8 observations. A sigmoidal fit was obtained for the data and the effective dose causing a 50% reduction of the maximum (ED$_{50}$) lung oedema was calculated. The statistical significance of differences between group data was determined using the Kruskal-Wallis multiple comparison test followed by a Dunnett’s post test with $P < 0.05$ accepted as significant ($* = P < 0.05$, ** = P < 0.01).

**Figure 4**
Effect of ciclesonide and fluticasone on adrenal weight following once daily, 7-day intratracheal treatment in Sprague-Dawley rats. Results are shown as the mean ± s.e. mean of n = 7-8 observations. The statistical significance of differences between group data was determined using the Kruskal-Wallis multiple comparison test followed by a Dunnett’s post test with $P < 0.05$ accepted as significant ($* = P < 0.05$, ** = P < 0.01).
Figure 5

Histograms illustrating the effect of ciclesonide and fluticasone on femoral growth plate width after 7-day treatment. Histograms depicting image analysis measurements of femoral head growth plate width from histological sections. Data are expressed as the percent decrease in femur growth plate width following 7-day intratracheal treatment compared with vehicle control. Results are shown as the mean ± s.e. of 7-8 rats. The statistical significance of differences between group data was determined using the Kruskal-Wallis multiple comparison test followed by a Dunnett’s post test with P < 0.05 accepted as significant (* = P<0.05, ** = P<0.01).

Figure 6.

Photomicrographs showing the proliferating zone of the femoral head growth plate and the effect on growth plate width following 7 day steroid treatment. Photomicrographs showing the proliferative zone of the femoral head growth plate and a typical steroid osteopenic reaction after vehicle (A), ciclesonide 0.1 mg/kg (B), fluticasone 0.1mg/kg (C) treatment for 7 days. Maturing cartilage can be seen above the growth plate, and cancellous bone with marrow cavities below. Tissue sections were stained with Alcian blue 8GX and counterstained with matoxylin and acid fuchsin/eosin mixture. Alcian blue-stained sections of decalcified femoral heads demonstrating the femoral head growth plate width. The dark proteoglycan-stained region indicates the proliferative zone, which is the region, measured to assess changes in femur growth plate width. Bars indicate 100 µm.
**Table 1:** Binding affinities of ciclesonide, *des*-CIC and fluticasone for the human glucocorticoid receptors.

<table>
<thead>
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<th>Test compounds</th>
<th>IC₅₀ (nM)</th>
<th>Ki (nM)</th>
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<td>0.31</td>
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<tr>
<td>Dexamethasone</td>
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<td>6.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>
**Figure 3**

% Inhibition of lung oedema vs. Log dose mg/kg i.t., -24 and 0 hrs

- Ciclesonide
- Fluticasone
Figure 4

The graph shows the relationship between the log dose (mg/kg day, i.t., 7 days) and the percentage of adrenal involution. The data points for Ciclesonide and Fluticasone are indicated with squares and diamonds, respectively. The trend lines for both groups show a decrease in adrenal involution as the log dose increases. The graph includes error bars and statistical significance symbols (***).
Figure 5

% Inhibition

<table>
<thead>
<tr>
<th>Compound dose (mg/kg/day), i.t., 7 days</th>
<th>0.01</th>
<th>0.1</th>
<th>1</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciclesonide</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Fluticasone</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

** indicates statistically significant difference.
Figure 6

A  

B  

C  

Vehicle  

Ciclesonide (0.1 mg/kg/day) Fluticasone (0.1 mg/kg/day)