The anti-inflammatory profile of inhaled corticosteroids: biopsy studies in asthmatic patients

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The beneficial effects of inhaled corticosteroids in the treatment of asthma are well established. A potent topical anti-inflammatory action is assumed to underlie the therapeutic effect, given that these agents alter the number and function of a range of inflammatory cells and markers in airway biopsies. This activity profile is shown by all inhaled corticosteroids, in a variety of patient types and study designs. Thus, treatment with inhaled corticosteroids leads to consistent reductions in the number and activation of mast cells and eosinophils in biopsy specimens. Other relevant findings include reductions in T-lymphocytes, which contribute to chronic inflammation via the secretion of pro-inflammatory cytokines (some of which are responsible for eosinophil accumulation and activation). Inhaled corticosteroids may therefore act by down-regulating immunoreactivity, so reducing activation of T lymphocytes and (consequently) eosinophils. There is considerable interest in whether corticosteroids can inhibit or reverse some structural changes in the airways, including basement membrane thickening, collagen deposition and increased airway vascularity; it has been suggested that these changes may contribute towards airway hyperresponsiveness and irreversible airway obstruction. In summary, inhaled corticosteroids have a broad spectrum of anti-inflammatory activity in asthma patients, but the relationship between changes in clinical and immunopathological parameters, particularly in the long-term, requires further study.

Key words: asthma; inhaled corticosteroids; anti-inflammatory agents; biopsy; airway remodelling; airway vascularity.

Introduction

Asthma is characterized pathologically by inflammation of the airways, associated with epithelial fragility and thickening of the reticular basement membrane, which in turn are thought to give rise to the clinical features of the disease (variable airway obstruction and hyperresponsiveness to various stimuli). The pivotal role of inflammation in the pathogenesis of asthma provides theoretical justification for the use of inhaled corticosteroids, which are known to exert a potent anti-inflammatory effect. These agents are therefore considered first-line therapy for the most patients with asthma (1,2), in the belief that if chronic inflammation can be suppressed then the associated short- and long-term consequences could be prevented. However, the mechanisms by which inhaled corticosteroids exert their anti-inflammatory activity are not fully understood. For example, it remains unclear which components of the inflammatory process are first affected by these agents, and which are most sensitive in terms of response to treatment.

This review discusses the anti-inflammatory profile of inhaled corticosteroids, with a view to further understanding their mechanism of action, based on the findings of endobronchial biopsy studies in patients with asthma.

Review of biopsy studies

EFFECT ON MAST CELLS AND EOSINOPHILS

It is well established that bronchial inflammation in asthma is characterized by increased numbers of mast cells, eosinophils and T-lymphocytes. Mast cells play an important role in the immediate asthmatic reaction to allergens (3), which is mediated by the release of powerful smooth muscle and vasoactive mediators such as histamine, prostaglandins and cysteinyl leukotrienes (4). Mast cell activation also leads to the release of chemoattractants for eosinophils; these cells release granular proteins (e.g. major...
basic protein and eosinophil cationic protein) that are toxic to the airway epithelium. The number of mast cells, eosinophils and T lymphocytes (along with the extent of their activation) has therefore been a major focus of endobronchial biopsy assessment in studies of inhaled corticosteroids, as discussed below.

Several inhaled corticosteroids are presently available, each of which has undergone evaluation in terms of endobronchial biopsy findings during treatment. For example, treatment with inhaled budesonide 800 μg twice daily was associated with a significant decrease in eosinophils in biopsy specimens from patients with mild asthma (5). A small placebo-controlled study of mildly asthmatic patients reported that 4 months of treatment with inhaled beclomethasone dipropionate (BDP) 500 μg twice daily led to significant reductions in the number of both mast cells and eosinophils, including the activated fraction (6). Hoshino et al. (7) also reported significant decreases in the number of activated eosinophils and mast cells in biopsy samples from patients treated with BDP for atopic asthma. These findings confirm the results of earlier studies, in which BDP significantly reduced the number of eosinophils in the epithelium and lamina propria of biopsy samples from asthmatic patients (8).

Fluticasone propionate (FP) is an inhaled corticosteroid that is highly effective in the treatment of asthma (9). Numerous studies have reported biopsy findings following treatment with this agent. In a placebo-controlled study of patients with mild asthma, for example, Li et al. reported that treatment for 6 weeks with inhaled FP 250 μg twice daily reduced the number of total eosinophils (EG1+) and mast cells by 53% and 29%, respectively (10). Similar findings were observed by Giszycki et al. (11) who examined the effect of FP 250 μg twice daily for 6 weeks on the late phase response to inhaled allergen; FP reduced numbers of eosinophils and mast cells in bronchial biopsies but an increased influx of neutrophils was also apparent, the clinical relevance of which is unclear. Olivieri et al. (12) also reported that treatment with inhaled FP was associated with a significant reduction in the number of eosinophils and mast cells (including the proportion of degranulated mast cells) in biopsy specimens, whereas placebo had no effect (Fig. 1). Evidence for an anti-inflammatory effect of FP was strengthened by the reductions in levels of mediators and numbers of activated cells in bronchoalveolar lavage (12).

Although inhaled corticosteroids are effective in the treatment of asthma, there is some evidence of ongoing airway inflammation despite maintenance therapy (13) which suggests that some components of airway inflammation are less sensitive to inhaled corticosteroids.

The effect of inhaled FP on airway inflammation at the maximum recommended dosage of 1000 μg twice daily was recently investigated in two studies (14,15). In the study by Booth et al. (14), in which patients with mild-to-moderate asthma received inhaled FP for 3 months, significant improvements in lung function were paralleled by a reduction of activated eosinophils (EG2+) in the lamina propria of bronchial biopsies. Differences in inflammatory indices from bronchoalveolar lavage were less conclusive, although a trend towards a reduction in eosinophils and mast cells was apparent (14). Lim et al. (5) reported similar findings in a study with budesonide: whilst eosinophil numbers in biopsy sections from patients with mild asthma were decreased, eosinophil counts in bronchoalveolar lavage were essentially unchanged. Faul et al. (15) were the first to test functional and immunological effects of an inhaled corticosteroid at successive timepoints within the same population of asthmatic patients. The numbers of EG1+ and EG2+ eosinophils in the bronchial wall were reduced from baseline within 2 weeks of commencing treatment with FP, accompanied by improvements in lung function. Thereafter, no further improvements in lung function were recorded. Immunohistologically, however, the improvement offered by FP was progressive; thus, the reduction in activated eosinophils (EG2+) was only significant after 8 weeks of treatment. The reasons for this complex relationship between improvement in lung function and immunological abnormalities during inhaled corticosteroid therapy are not fully understood; however,
the reduction in airway inflammation after 2 weeks may correlate with other outcome measures such as bronchial hyperresponsiveness or asthma exacerbations.

Although not a biopsy study, the findings of Meijer et al. (16) add to our understanding of the effect of inhaled corticosteroids on airways inflammation. These authors used the technique of induced sputum, in which patients inhale nebulized hypertonic saline and are then encouraged to cough and expectorate sputum, to compare the effects of inhaled FP (at dosages of 500 and 200 µg day\(^{-1}\)) versus oral prednisolone (30 mg day\(^{-1}\)) on airways inflammation. Overall, there was a dose-dependent reduction in eosinophil numbers and eosinophil cationic protein levels in sputum after 2 weeks of treatment with inhaled FP. At the higher FP dosage, these changes were similar to those observed for oral prednisolone (Fig. 2), and were accompanied by improvements in lung function and hyperresponsiveness that were significantly greater than those in the prednisolone group (16).

**EFFECT ON T LYMPHOCYTES**

The chronic airway inflammation in patients with asthma is also associated with prolonged residence in the bronchial wall of activated T-lymphocytes (17). These cells therefore represent further targets for therapeutic intervention, and the effect of inhaled corticosteroids on T-lymphocytes has been evaluated in several biopsy studies in asthmatic patients (10,14,15). For example, Faul et al. (15) reported that 8 weeks of treatment with inhaled FP (1000 µg twice daily) produced reductions in the number of T-lymphocytes and CD45RO+ T-lymphocytes in the bronchial wall within 2 weeks of commencing therapy. Given the concomitant profile of reductions in eosinophils over the 8-week treatment period (as described above), the authors speculated that the reduction in eosinophils occurred as a consequence of the initial decrease in the number of CD45RO+ T-lymphocytes in the mucosa. This is in accordance with the knowledge that T-cell-derived cytokines, such as interleukin-5 and granulocyte macrophage-colony-stimulating factor (GM-CSF), are responsible for accumulation and prolonged survival of eosinophils in the airways (18,19).

The primary mechanism of action of inhaled corticosteroids may therefore be to down-regulate immunoreactivity, thereby reducing T-lymphocyte function and, consequently, eosinophil activation. This effect may be dose-dependent, given that studies with lower doses of inhaled corticosteroids (even allowing for differences in potency) failed to demonstrate an effect on helper or activated helper T-cells despite reductions in total and activated eosinophils (6). Indeed, Bamford et al. (20) recently reported that although inhaled corticosteroid therapy, in this case BDP (200–1500 µg day\(^{-1}\)), can effectively normalize elevated numbers of mast cells and eosinophils in airway biopsies, patients may remain symptomatic. Other studies with BDP, however, reported significant decreases in CD3+ and CD4 + T-cells during 12 weeks of treatment, which were associated with significant improvement in airway responsiveness in patients with atopic asthma (7). It is interesting to speculate whether the absence of an effect on T-lymphocytes may explain continuing symptoms in some asthmatic patients despite inhaled corticosteroid therapy, since recent studies show that persistent T-cell activation is a prominent feature of severe asthma (21). Interestingly, Vrugt et al. (21) also showed the absence of prominent mucosal eosinophilia in patients with severe asthma, indicating the likely contribution of other inflammatory cells or mechanisms to the continuing disease in these patients. Possible mechanisms are discussed in the following sections.

**EPITHELIUM**

Bronchial epithelium plays an important role in the regulation of airway function (22). For example, epithelial cells are damaged and shed during asthmatic exacerbations, leading to increased permeability of airways to inhaled...
allergens and exposure of afferent nerve endings. The release of sensory neuropeptides by such airway neurones is thought to contribute to ongoing inflammation, bronchoconstriction, microvascular leakage and secretion of mucus. Epithelial damage is probably mediated via the release of basic proteins from activated eosinophils and proteolytic enzymes from mast cells, leading to the release of a range of mediators, cytokines, chemokines and growth factors that act as chemotaxants, inhibit apoptosis of eosinophils and stimulate expression of cellular adhesion molecules. Maintenance of epithelial integrity and function therefore represents a target for anti-asthma therapy. There is evidence to suggest that inhaled corticosteroids reduce epithelial damage and shedding, as shown by a reduction in epithelial cell counts in bronchoalveolar lavage following treatment with inhaled corticosteroids (14,23). This has also been demonstrated in biopsy studies, in which 10 years of treatment with inhaled corticosteroids was associated with improvements in epithelial abnormalities (24).

AIRWAY REMODELLING

A large body of evidence supports the fact that asthma is a chronic inflammatory disorder of the airways involving release of mediators from both mast cells and eosinophils, and orchestrated by T-lymphocytes. A feature of the chronic inflammatory process in asthma is airway remodelling (25), which is characterized by extensive deposition of collagen beneath the sub-epithelial layer. Fibroblasts appear to play an important role in this thickening of the basement membrane (26) which, in conjunction with smooth muscle hypertrophy/hyperplasia, has been suggested to contribute towards persistent hyperresponsiveness and irreversible obstruction of the airways (27). Indeed, increased basement membrane thickness has been shown to be positively correlated with airway reactivity to methacholine and negatively correlated with forced expiratory volume in 1 sec (FEV1) (26,28); however, Chu et al. showed no correlation between basement membrane thickness and asthma severity (29).

A number of studies have investigated whether inhaled corticosteroids can alter the thickness of the basement membrane. In a small group of patients with mild asthma, no alteration in thickness was seen after treatment with budesonide 200 µg for 4 weeks (13). Although the study by Trigg et al. suggested that treatment with BDP for 4 months reduced basement membrane thickness, this was measured using an antibody to a collagen component rather than by the conventional method of light or electron microscopy (6). Olivieri et al. have also suggested that fluticasone propionate may reduce basement membrane thickness, but differences in the baseline measurements of thickness between the treated and placebo groups confound interpretation of this study (12). Beneficial effects of an inhaled corticosteroid were reported by Laitinen et al. who showed that the thickness of the tenascin layer in the basement membrane of patients with seasonal asthma fell by 39% after treatment with budesonide for 4-6 weeks during the pollen season (30). More studies are needed before a definite effect of inhaled corticosteroids on basement membrane thickening can be proven.

Increased vascularity is another aspect of the structural remodelling that occurs during chronic inflammation of the airways (31), and may have several consequences for patients with asthma. The most notable is a possible effect on airway responsiveness, since a small increase in thickness of the airway wall (e.g. because of oedema and/or microvascular engorgement) may lead to pronounced narrowing of the airway lumen during smooth muscle contraction (32). As described above, inhaled corticosteroids potentially reduce airway remodelling although data pertaining to their effect on increased airway vascularity are limited. In one recent study, Orsida et al. (33) reported a significant increase in the density of vessels in the lamina propria of mildly asthmatic patients who were not receiving inhaled corticosteroids compared with non-asthmatic controls, and vessel density was strongly correlated with both lung function and hyperresponsiveness. Interestingly, those patients being treated with an inhaled corticosteroid showed a decreased level of vascularity (versus those not on such treatment), and this effect showed a trend towards dose-dependency. Reduction of airway wall vascularity may therefore contribute to the therapeutic efficacy of inhaled corticosteroids, particularly at high dosages. Further studies are clearly warranted to delineate the mechanism of this anti-angiogenic effect.

Conclusions

The range of anti-inflammatory properties demonstrated for inhaled corticosteroids in biopsy studies on asthmatic patients can be summarized as follows: reduction in numbers of mast cells (most pronounced in atopic patients); reduction in numbers and activation of eosinophils; reduction in numbers and function of T-lymphocytes (particularly CD4+ cells); improved epithelial morphology and reduction in epithelial cell activation, and some degree of reversal of airway remodelling. The combination of activities has been shown consistently among different patient types and in a variety of study designs.

Overall, these findings indicate that inhaled corticosteroids have a broad spectrum of anti-inflammatory effects, which supports their use as first-line therapy for asthma. Further studies are required to investigate the mechanism of continued inflammation in severe asthma, and the effect of inhaled corticosteroids on structural changes in the airways and how these changes relate to chronic inflammation.

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