

Pathophysiological effects of glucocorticoids on nasal polyps: an update

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Purpose of review

The exact mechanisms by which glucocorticoids exert their beneficial effects on nasal polyps are not clearly defined. Nasal polyps, asthma and allergic rhinitis share common features such as mucosal infiltration with eosinophils and mast cells as well as local IgE production. The present review is an update on the pathophysiological mechanisms of glucocorticoids on nasal polyps described during the last 2 years.

Recent findings

The reduction of leukocyte numbers in nasal polyps following glucocorticoid treatment depends on several mechanisms, for example altered balance between the two isoforms of the human glucocorticoid receptors, GR α and GR β . Another explanation may be inhibition of CD4+ T by CD8+ T cells. Increased expression of the antiinflammatory cytokine transforming growth factor β may contribute to this. A DNA microarray study which examined the expression of some 22 000 genes showed increased expression of several antiinflammatory genes in nasal polyps after treatment with glucocorticoids. The antiinflammatory gene that increased most was uteroglobin (also known as Clara cell protein 16) which is abundantly expressed in airway secretions and thought to have an important role in regulating inflammation.

Summary

Glucocorticoids affect both pro and antiinflammatory pathways in nasal polyps. Upregulation of antiinflammatory genes such as transforming growth factor β and uteroglobin may play an important role. Elucidation of these mechanisms may help us to understand not only the effects of glucocorticoids on nasal polyps, but also on related disorders such as allergic rhinitis and asthma.

Keywords

glucocorticoids, microarray, nasal polyps

Abbreviations

MCP monocyte chemotactic protein
TGF transforming growth factor

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Introduction

Allergic rhinitis, asthma and nasal polyps are characterized by an eosinophilic edematous stroma [1]. Immunohistochemical investigations have revealed mast cell degranulation and high local levels of IgE [2^{••},3]. In addition to their histological similarities, all three diseases usually respond favourably to treatment with glucocorticoids. The pathophysiological mechanisms that underlie these responses have not been clearly defined. Nasal polyposis has advantages as an in-vivo model to study the effects of local treatment with glucocorticoids. In contrast to, for example, bronchial tissue from patients with asthma, biopsies are relatively easily obtained from nasal polyps. In fact, whole polyps may be surgically removed for therapeutic reasons. Studies of glucocorticoid-treated nasal polyps have demonstrated the down-regulation of proinflammatory cytokines and adhesion molecules that attract and activate eosinophils [2^{••},4]. Studies of asthma and allergic rhinitis show that glucocorticoids decrease a wide variety of proinflammatory cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-11, IL-13, tumour necrosis factor α , granulocyte/macrophage colony-stimulating factor), chemokines [IL-8, RANTES, macrophage inflammatory protein-1 α , monocyte chemoattractant protein (MCP)-1, MCP-3, MCP-4, eotaxin], adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, E-selectin) and mediator-synthesizing enzymes (inducible nitric oxide synthase, cyclooxygenase-2, phospholipase-A2) [5^{••},6].

These findings lead to the question of how many genes there are whose expression is affected by glucocorticoids. DNA microarray technology allows simultaneous analysis of expression of the whole human genome. This provides unique opportunities to analyse the effects of glucocorticoids on a genome-wide scale [7]. Studies of this kind have been performed on a variety of cell types [8–12]. They reveal that glucocorticoids affect expression of hundreds of genes. Not only do glucocorticoids downregulate proinflammatory mediators but they also upregulate antiinflammatory agents. Furthermore, gene

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expression profiles vary considerably in different cell types. This is consistent with previous data. For example, glucocorticoids induce apoptosis in eosinophils but inhibit apoptosis in neutrophils [13]. Different doses or types (i.e. natural or synthetic) of glucocorticoids could also contribute to the variety of gene expression profiles. Taken together, these data indicate that the effects of glucocorticoids depend on altered expression of hundreds of genes, which may differ in different cell types depending on which type of glucocorticoid is used. To understand the clinical effects of glucocorticoids it may therefore be useful to study human inflamed tissue treated with pharmacological doses of glucocorticoids. Nasal polyps are particularly suited for this given their accessibility, the amount of tissue that can be obtained and the fact that they often respond well to treatment with glucocorticoids.

Effects of glucocorticoids on cellular composition and activation

The cellular composition of nasal polyps may be seen as the net result of complex and interacting mechanisms that regulate influx, proliferation and survival of cells. Recent studies have increased our understanding of how glucocorticoids affect these mechanisms. Interestingly, one example is based on a clinical observation. Not all patients with nasal polyps respond to glucocorticoids. A possible explanation for this could be alterations in the relative expression of the two isoforms of the human glucocorticoid receptor, GR α and GR β . Increased expression of GR β has been reported in patients with glucocorticoid-resistant asthma, ulcerative colitis and nasal polyposis [14,15]. Downregulation of GR α after treatment with glucocorticoids has also been reported and could explain secondary glucocorticoid resistance [16]. In a recent study, Pujols *et al* [17 $\bullet$], found that expression of GR β was higher in nasal polyps than in nasal mucosa and correlated with increased infiltration of inflammatory cells. These findings suggest the value of linking clinical observations and research.

Influx of inflammatory cells depends on adhesion molecules, chemokines and cytokines. Decreased expression of these molecules has previously been shown [4]. However, Papon *et al.* [18] did not find decreased expression of intercellular adhesion molecule-1 in epithelial cells from nasal polyps.

Th2 cytokines released from CD4 $^{+}$ T cells induce recruitment of eosinophils, which comprise more than 60% of the cell population with nasal polyps, except in cystic fibrosis [2 $\bullet\bullet$]. CD4 $^{+}$ T cells are thought to be counter-regulated by CD8 $^{+}$ T cells. It is not clear how this negative feedback mechanism is mediated. Transforming growth factor β (TGF- β) is an antiinflammatory cytokine that may have a key role in this context. In an elegant study on nasal polyps, Mastruzzo *et al.* [19 $\bullet\bullet$]

found that intranasal glucocorticoids increased the density of CD8 $^{+}$ T cells in nasal polyps. Moreover, the CD4/CD8 ratio decreased. This was accompanied by a decrease in IL-4 $^{+}$ and IL-5 $^{+}$ cells. By contrast, a marked increase in TGF- β $^{+}$ cells was noted. These cells were positively correlated with CD8 $^{+}$ T cells, but negatively correlated with IL-4 $^{+}$ cells. The authors suggested that the increase in CD8 $^{+}$ T cells and TGF- β $^{+}$ cells could be mechanistically linked and may explain the decrease in the CD4/CD8 cells and Th2 cytokines. This is the first study to show this *in vivo* and supports the finding that the antiinflammatory effect of TGF- β contributes to the beneficial effects of glucocorticoids [19 $\bullet\bullet$]. Increased expression of TGF- β , however, may not be entirely beneficial. In addition to its antiinflammatory effect, TGF- β also upregulates collagen synthesis in fibroblasts. This may cause lung fibrosis in humans and experimental animal models [20]. However, Mastruzzo *et al.* [19 $\bullet\bullet$] only found weak staining for collagen in glucocorticoid-treated nasal polyps. This finding agrees with studies of patients treated with high doses of glucocorticoids, in which no evidence of fibrosis was found [21]. This is further supported by a recent report where glucocorticoids were found to decrease proliferation of cultivated fibroblasts from nasal polyps [22]. The authors actually suggested that reduced proliferation of fibroblasts in polyps could contribute to the beneficial effects of treating nasal polyps with glucocorticoids. Decreased cellular infiltration in nasal polyps could also depend on apoptosis. Indeed, Watanabe *et al.* [23] found that glucocorticoids induce apoptosis of eosinophils and T lymphocytes both *in vivo* and *in vitro*.

Mastruzzo *et al.* [19 $\bullet\bullet$] also investigated the epithelium in nasal polyps before and after treatment. Untreated, the epithelium of polyps was substantially damaged. Treatment with glucocorticoids resulted in remarkable reconstitution of epithelial cells [19 $\bullet\bullet$].

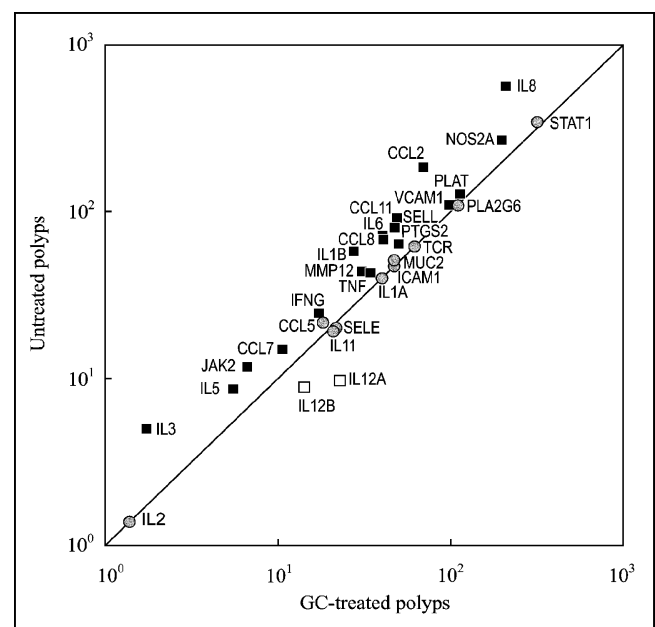
Effects of glucocorticoids on antiinflammatory mediators

The study by Mastruzzo *et al.* showing decreased expression of TGF- β is conceptually interesting in that it implies that glucocorticoids may act by upregulating antiinflammatory genes rather than only downregulating proinflammatory genes. The effects of glucocorticoids on antiinflammatory genes are poorly defined. DNA microarrays allow analysis of how glucocorticoids affect expression of the whole human genome (Fig. 1). In principle this technology is based on attaching short DNA sequences corresponding to known genes on a miniature glass surface. These sequences can hybridize to complementary mRNA from cells or tissues. The relative abundance of such transcripts can then be measured. At present there are DNA microarrays with DNA sequences corresponding to more than 40 000 genes and variants

Figure 1. A DNA microarray GeneChip

These can be used to analyse up to 44 000 transcripts (image courtesy of Affymetrix).

attached to the same surface. DNA microarray studies of the effects of glucocorticoids on a variety of cell types have been performed. These studies have shown altered expression of many antiinflammatory genes. However, gene expression profiles vary considerably between different cell types [8–12]. This is consistent with previous studies, but interpretation of the results is complicated by the cells being activated by different agents in different doses. These agents only partially reflect in-vivo conditions. Furthermore, different doses and types of glucocorticoids were used. The first DNA microarray analysis of human tissue treated with pharmacological doses of glucocorticoids was recently performed on nasal polyps [24]. This study showed that some 200 genes (out of 22 000 analysed genes) changed in expression following glucocorticoid treatment. Genes associated with inflammation comprised the largest functional group. Several genes previously known to be downregulated by glucocorticoids also showed lower expression in treated polyps (Fig. 2). In addition, several proinflammatory genes that had not been previously described as glucocorticoid regulated were downregulated. These genes were involved in key steps of inflammation (e.g. immunoglobulin production; antigen processing and presentation; the chemoattraction and activation of granulocytes and lymphocytes). The main finding, however, was the up-regulation of antiinflammatory genes. The gene that increased most in expression was uteroglobin (also known as Clara cell protein 16, CC16).

Figure 2. Expression levels of genes previously described as downregulated by glucocorticoids in nasal polyps before and after treatment with a local glucocorticoid (GC)

The diagonal line is the line of identity. Ten of the 30 genes differed less than 10% (circles), and of the remaining 20, two had a higher (open boxes) and 18 a lower expression (filled boxes) after treatment ($P = 0.001$ with Wilcoxon's signed rank test) [24].

Other antiinflammatory genes that increased include arachidonate 15-lipoxygenase. This is an enzyme that induces production of lipoxins, a class of eicosanoids that are important stop signals for inflammatory reactions [25]. Other upregulated antiinflammatory genes included IK-cytokine, which decreases major histocompatibility complex (MHC) class II expression. This is consistent with the lower expression of MHC class II that was demonstrated in the glucocorticoid-treated polyps. Similarly, the increased expression of CD24, which induces B-cell apoptosis, agrees with the reduced expression of genes expressed by B cells. These findings support the hypothesis that induction of antiinflammatory mediators may contribute to the beneficial effects of glucocorticoids in airway inflammation. Of these antiinflammatory genes uteroglobin may be of particular relevance. Experiments show that uteroglobin synthesis is induced by glucocorticoids [26]. It has wide-ranging antiinflammatory effects, that is the inhibition of leukocyte chemotaxis, phospholipase-A2 and proinflammatory cytokines, as well as protease activity. Peptides derived from uteroglobin are among the most potent antiinflammatory agents identified to date. Recently, a peptide of this kind was successfully tested in an animal model of allergic conjunctivitis [27]. Moreover, compared with healthy controls, lower expression of uteroglobin has been found not only in nasal polyps but also in asthma [28]. Taken together the data support the finding that

uteroglobin may play a role in the pathogenesis of nasal polyps and asthma.

In another microarray study that analysed nasal mucosa from patients with allergic rhinitis with and without nasal polyps, increased expression of mammaglobin B was found in the patients with polyps [29]. This is of interest since mammaglobin and uteroglobin belong to the same gene family – the secretoglobins. Although the biological role of mammaglobin is not known, these findings suggest that DNA microarrays could help detect differences in gene expression in diseases with similar histopathology such as allergic rhinitis and nasal polyposis. These differences may help elucidate pathogenic mechanisms, as well as finding markers to predict and monitor response to treatment with glucocorticoids. The fact that glucocorticoids upregulate expression of uteroglobin in nasal polyps suggests that this protein may have potential as a marker to monitor response to glucocorticoids. It is of note in this context that lower levels of uteroglobin in patients with asthma have been linked to polymorphisms in this gene [28]. Since uteroglobin appears to be involved in the pathogenesis of nasal polyposis it would be of interest to examine if the same polymorphism also occurs in nasal polyposis, and if so whether it is linked to variable responses to glucocorticoids. Eosinophilic nasal polyposis may be well suited for such studies because it is a disease that has a hereditary component and it can be clearly defined phenotypically. Interestingly, many studies have focused on finding candidate genes for asthma, but none in nasal polyposis.

Conclusion

Glucocorticoids have complex effects on cellular composition and activation in nasal polyps. Considering the value of nasal polyposis as an in-vivo model of how inflamed tissue responds to treatment with glucocorticoids, surprisingly few studies about this have been published during the last 2 years. However, the published studies show that decreased infiltration of leukocytes may depend on several mechanisms, such as altered proportion between glucocorticoid receptors α and β . Increased expression of antiinflammatory genes, for example TGF- β and uteroglobin, may also contribute. These findings lead to questions that may be of interest for future studies: (1) Are the genes that change following treatment with glucocorticoids also involved in the pathogenesis of nasal polyposis? (2) Could variable effects of glucocorticoids on these genes explain why some patients respond less favourably to treatment? (3) Can DNA microarray analyses of nasal polyps help identify mediators that are specific for such patients? (4) Could polymorphisms in genes such as uteroglobin explain variable responses to glucocorticoids? Questions (3) and (4) lead on to an area of research with considerable clinical relevance – pharmacogenomics. This discipline aims to

identify markers to predict drug response. By using such markers clinicians may more accurately select the appropriate drug for individual patients. Examination of gene polymorphisms and expression profiles in glucocorticoid-treated nasal polyps could prove to be a well defined model for pharmacogenomic studies. (5) Could antiinflammatory genes that increase after glucocorticoid treatment be of use therapeutically, without the side effects of glucocorticoids? (6) To what extent do these questions relate to other airway diseases such as allergic rhinitis and asthma? Taken together these questions indicate that coming studies on the effects of glucocorticoids on nasal polyps may prove rewarding.

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