Inhibition of GM-CSF secretion by topical corticosteroids and nedocromil sodium. 
A comparison study using nasal polyp epithelial cells

J. MULLOL*, J. ROCA-FERRER*, A. XAUBET*, J. TRASERRA‡ and C. PICADO*

*IDIBAPS, †Institut de Pneumologia i Cirurgia Toràcica, ‡Servei d’Otorino-laringologia, Hospital Clínic, Departament de Medicina, Universitat de Barcelona, Spain

Nasal epithelial cells maintain eosinophil survival by secreting granulocyte/macrophage colony-stimulating factor (GM-CSF). Corticosteroids antagonize eosinophil viability induced by GM-CSF. We investigated the effect of topical corticosteroids and nedocromil sodium on the release of GM-CSF from nasal polyp epithelial cells.

Epithelial cells were obtained from 19 patients undergoing nasal polypectomy and cultured. After reaching confluence, cultured cells were stimulated with 10% foetal calf serum in the absence and presence of four topical corticosteroids and nedocromil sodium for 48 h. GM-CSF was measured by enzyme linked immunosorbent assay (ELISA).

Fluticasone propionate was the most potent inhibitor of GM-CSF release (IC25 = 46 pM) closely followed by budesonide (IC25 = 4 nM), beclomethasone dipropionate (IC25 = 40 nM) and triamcinolone acetonide (IC25 = 75 nM). Nedocromil sodium had no effect on GM-CSF release.

We conclude that the effect of topical steroids on reducing eosinophil infiltration in nasal polyps may be due in part to downregulation, among other cytokines, of epithelial GM-CSF production which prolongs eosinophil viability. Quantitatively, fluticasone propionate inhibited GM-CSF production more potently than budesonide, beclomethasone dipropionate and triamcinolone acetonide.

Key words: corticosteroids; eosinophil; GM-CSF; nasal polyps

Introduction

There is evidence that the eosinophil infiltrate in nasal polyps is the consequence of both preferential recruitment of eosinophils (1,2) and prolonged eosinophil survival within the inflamed microenvironment (3,4).

Epithelial cells synthesize a number of growth factors and cytokines (5). Among other potential cytokines, nasal epithelial cells maintain eosinophil survival by secreting granulocyte/macrophage colony-stimulating factor (GM-CSF) (2,3,4). Corticosteroids antagonize the viability-sustaining effect of GM-CSF (3,4).

Topical corticosteroids are the recommended treatment for patients with nasal polyposis (6). Nasal polyp epithelial cells are the first line target for topical corticosteroids. In this study we focused on the effect of topical corticosteroids on GM-CSF because of the important role of this cytokine in eosinophil survival. We hypothesize that topical corticosteroid potency should be related to their capacity to inhibit the release of GM-CSF.

Methods

SUBJECTS

Nasal polyps were obtained from 19 patients (12 men, seven women), aged 50 ± 2 years, undergoing nasal polypectomy. Seventeen patients (90%) were on intranasal or oral corticosteroids at the time polypectomy was carried out. The study was approved by the Ethics Committee of our Institution.

EPITHELIAL CELL CULTURE

Transport of nasal polyps, and isolation, characterization and culture of epithelial cells, were carried out according to a method previously described in detail (3). After reaching confluence, cultured epithelial cells were incubated with 10% foetal calf serum (FCS, Flow laboratories) for 48 h in the absence (control) and presence of budesonide (Astra, Barcelona, Spain), fluticasone propionate (Glaxo Wellcome, Greenford, U.K.), beclomethasone dipropionate (Glaxo...
Wellcome, Greenford, U.K.), triamcinolone acetonide (Sigma, Spain) and nedocromil sodium (Fisons Ibérica, Zaragoza, Spain). Dose-response curves were obtained using concentrations from $10^{-5}$ to $10^{-13}$ M.

**GM-CSF PRODUCTION**

GM-CSF concentration in the cell supernatants was directly measured by a commercial enzyme-linked immunoassay using a ‘sandwich’ technique (Amersham Ibérica, Spain). GM-CSF limit of detection was 4 pg/ml.

**STATISTICAL ANALYSIS**

Statistical evaluation was performed using the statistical software package Statview II (Brainpower Inc. U.S.A.). Results are expressed as mean ± standard error of the mean (SEM). A non-parametric test (Wilcoxon) was used for statistical comparisons. A $P<0.05$ was considered statistically significant.

**Results**

Incubation of nasal polyp epithelial cells with 10% FCS increased GM-CSF production in all experiments. At baseline, the range of GM-CSF secretion was 26–89 pg ml$^{-1}$ while after 10% FCS stimulation was 213–684 pg ml$^{-1}$. FCS-induced release of GM-CSF was inhibited in a dose-dependent manner by all four topical corticosteroids. The inhibitory effects at $10^{-5}$ M concentration were: fluticasone propionate 71% ($n=6$, $P<0.01$), budesonide 58% ($n=7$, $P<0.01$), triamcinolone acetonide 42% ($n=6$, $P<0.02$) and beclomethasone dipropionate 40% ($n=6$, $P<0.02$), compared to positive control (10% FCS-treated cells).

The minimal inhibitory concentration was $10^{-11}$ M for fluticasone and $10^{-9}$ M for the other three corticosteroids.

![Fig. 1. Effect of topical corticosteroids on GM-CSF production from nasal polyp epithelial cells. Foetal calf serum (FCS) at 10% increased GM-CSF production compared to media-treated cells (control). Fluticasone propionate (FLU, $n=6$), budesonide (BUD, $n=7$), beclomethasone dipropionate (BDP, $n=6$) and triamcinolone acetonide (TRI, $n=6$) caused a dose-related inhibitory effect on FCS-induced GM-CSF release. Values are expressed as mean ± SEM. Wilcoxon signed-rank test (**$P<0.01$, compared to media-treated cells; †$P<0.05$ and ††$P<0.01$, compared to FCS-treated cells).](image-url)
Table 1. Inhibitory concentration of 25% (IC$_{25}$) of topical glucocorticoids and nedocromil sodium on GM-CSF release induced by 10% fetal calf serum in cultured nasal epithelial cells from nasal polyps.

<table>
<thead>
<tr>
<th>Anti-inflammatory drugs</th>
<th>IC$_{25}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluticasone propionate</td>
<td>46 ± 35 pm$^*$</td>
</tr>
<tr>
<td>Budesonide</td>
<td>4 ± 2 nm$^*$</td>
</tr>
<tr>
<td>Beclomethasone dipropionate</td>
<td>40 ± 17 nm$^*$</td>
</tr>
<tr>
<td>Triamcinolone acetonide</td>
<td>75 ± 42 nm$^{1,5}$</td>
</tr>
<tr>
<td>Nedocromil sodium</td>
<td>&gt; 10 μM$^*$</td>
</tr>
</tbody>
</table>

Wilcoxon Signed-Rank test ($P < 0.05$): $^*$compared to fluticasone propionate; $^{1,5}$compared to budesonide; $^*$compared to all glucocorticoids.

(Fig. 1). Nedocromil sodium at 10$^{-5}$ M did not have a significant inhibitory effect on GM-CSF production (10%, n = 6).

The potencies of the four corticosteroids on GM-CSF release were compared. Table 1 shows the concentration of corticosteroids producing 25% inhibition (IC$_{25}$) of GM-CSF release. We used IC$_{25}$ instead of IC$_{50}$ because most corticosteroids did not reach 50% inhibition of the FCS-induced GM-CSF production. Fluticasone propionate was the most potent inhibitor of GM-CSF release, and was significantly more potent than budesonide. Both fluticasone propionate and budesonide were more potent than beclomethasone dipropionate and triamcinolone acetonide. All glucocorticoids were more potent than nedocromil sodium.

Discussion

In this study we investigated the ability of several topical corticosteroids and nedocromil sodium to modulate the release of GM-CSF from nasal polyp epithelial cells in vitro. However, the role of cytokines produced by epithelial and other cells, such as IL-5 and eotaxin, which are potent eosinophil modulators should not be overlooked.

The effect of topical corticosteroids on GM-CSF release from nasal polyp epithelial cells has significance because previous studies have shown that GM-CSF is responsible for the induction of eosinophil survival (3,4). The chronic presence of eosinophils in the respiratory mucosa results in epithelial damage caused by cytotoxic products released by degranulated cells. By reducing eosinophil viability, topical corticosteroids contribute to reduce inflammation and therefore facilitate mucosal damage repair. In this regard, Kanai et al. (7) have shown the efficacy of budesonide in reducing the number of activated eosinophil in nasal polyps.

Our in vitro assay of topical corticosteroid potency tested four topical corticosteroids for their capacity to inhibit the release of GM-CSF from nasal polyp epithelial cells. The comparison study showed that fluticasone propionate is the most potent, followed by budesonide, beclomethasone dipropionate and triamcinolone acetonide. Nedocromil sodium had no effects on GM-CSF secretion.

The rank of potency found in our study closely agrees with the rank order and relative potency obtained with the same corticosteroids using the classical skin blanching method. With this method the potency of fluticasone was 1200 (relative to the potency of dexamethasone, considered 1), budesonide 980, beclomethasone dipropionate 600 and triamcinolone acetonide 330 (8).

Ideally, the validity of results obtained in in vitro studies should be reproduced in in vivo surveys. Long-term treatment with topical corticosteroids has been shown to reduce symptoms of nasal polyposis and reduce polyp recurrence following surgery (9-11). However, only a few clinical trials have compared the clinical response of nasal polyposis to different corticosteroids.

Two recent studies compared fluticasone propionate and beclomethasone dipropionate in patients with severe polyposis (12,13). In the two studies there was evidence that patients treated with fluticasone propionate responded faster, and that the magnitude of the response was greater than in patients receiving beclomethasone dipropionate. Although our in vitro model may have some limitations in relation to the presence of a constant steroid concentration, the absence or decrease of metabolism, and the potential lack of active metabolite formation, for instance beclomethasone 17-monopropionate from beclomethasone dipropionate, the present in vitro model may be used as a useful method to investigate and compare the potency of topical anti-inflammatory drugs.

Although previous studies have shown that nedocromil sodium significantly prevents stimulated GM-CSF production in both cultured human bronchial (14) and nasal epithelial cells (15), we could not find any significant effect of this drug on GM-CSF release from cultured nasal polyp epithelial cells. This finding suggests that the inflamed microenvironment of nasal polyps had induced a relative resistance to the pharmacological activity of nedocromil sodium in epithelial cells. To our knowledge, nedocromil sodium has not been used in the treatment of nasal polyposis. However, our results predict poor clinical results for this drug in the treatment of nasal polyps.

In summary, our study suggests that topical steroids may reduce eosinophil infiltration in nasal polyps by down-regulating the production of epithelial cell-derived cytokines, such as GM-CSF, which prolongs eosinophil viability. Quantitatively, the following rank of potency was observed: fluticasone propionate > budesonide > beclomethasone dipropionate > triamcinolone acetonide > nedocromil sodium.

Acknowledgements

This study was supported by grants from Sociedad Española de Neumología y Cirugía Torácica (SEPAR),
References


