

Original article

Topical glucocorticoids downregulate COX-1 positive cells in nasal polyps

Background: Influx of inflammatory cells is one of the hallmarks of nasal polyposis. As glucocorticoids (GC) are known to exhibit strong anti-inflammatory effects, these drugs are frequently used in the treatment of the disease. Part of the anti-inflammatory effects of GC is attributed to their interference with prostanoid synthesis. As cyclooxygenases (COX) are key enzymes in the synthesis of both pro- (COX-1, COX-2) and anti-inflammatory prostanoids (COX-2), we investigated the role of topical GC on COX-1, COX-2 and inflammatory markers in nasal polyps (NP).

Methods: Immunohistochemical analysis of inflammatory markers (CD68, CD117, MBP, elastase, IgE, BB-1, IL-4, IL-5 and IL-6), COX-1 and COX-2 was performed on normal nasal mucosa (NM) ($n = 18$), non-GC treated NP ($n = 27$) and topical GC treated NP ($n = 12$). NP groups were matched for allergy, asthma and ASA intolerance.

Results: Increased numbers of eosinophils, IL-5+ cells and IgE+ cells and decreased numbers of mast cells are striking features of NP inflammation ($P < 0.05$). In addition, increased numbers of COX-1+ cells are observed in NP epithelium compared to NM ($P < 0.05$).

Conclusion: Topical GC significantly reduce the number of COX-1+ NP cells ($P < 0.05$), but have no significant effect on COX-2+ NP cells. No significant reduction in the number of eosinophils is observed for GC treated NP. The number of IL-5+ cells is however increased significantly upon GC treatment ($P < 0.05$).

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Nasal polyposis is a multifactorial disease, associated with asthma, primary ciliary dyskinesia, acetyl salicylic acid (ASA) intolerance and possibly allergy, affecting between 1% and 4% of the general population (1, 2). Although many hypotheses have been suggested, the pathogenesis of nasal polyposis is still largely unknown. Macroscopically, nasal polyps are characterized by the presence of edematous masses of inflamed mucosa that are predominantly localized in the middle meatus and prolaps into the nose, leading to nasal obstruction, secretion, loss of smell, headache/ facial pain, and reduced quality of life (3, 4). Histologically, nasal polyps are characterized by a cover of respiratory epithelium, large quantities of extracellular oedema and a dense inflam-

matory cell infiltrate consisting of mast cells, eosinophils, lymphocytes, neutrophils and plasma cells, that release a variety of pro-inflammatory mediators, including cytokines, histamine, prostanoids and leukotrienes [for review see Fokkens et al. (3)].

In recent years, various randomized controlled trials have demonstrated the effectiveness of topical glucocorticoids (GC) in the treatment of primary and recurrent nasal polyposis (5–13). However, the mechanisms underlying the anti-inflammatory and immunoregulatory effects of these drugs *in vivo* remain to be fully explained.

GC are known to exhibit anti-edematous and strong anti-inflammatory effects that are in part attributed to their interference with prostanoid synthesis (14). Cyclooxygenases are key enzymes in the generation of prostanoids from arachidonic acid and exist in at least two isoforms: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 is considered to be expressed constitutively in all cells, whereas COX-2 is considered to be induced under inflammatory conditions. As both isoforms

Abbreviations: AP, alkaline phosphatase; ASA, acetyl salicylic acid; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; ENT, ear, nose and throat; ESS, endoscopic sinus surgery; GC, glucocorticoids; NP, nasal polyps; NM, normal nasal mucosa; NSAIDs, nonsteroidal anti-inflammatory drugs; PBS, phosphate-buffered saline.

are expressed in nasal polyposis and as prostanoids are known to control chemotaxis of inflammatory cells, vascular tone, vascular permeability and mucous secretion (all hallmarks of nasal polyposis) (15, 16), their possible involvement in the pathogenesis of nasal polyposis has long been studied. As COX-2 is presumed to be induced under pro-inflammatory conditions, most studies focussed on the role of this enzyme in the pathogenesis of nasal polyposis. Recent studies indicate however, that mediators generated by COX-2 can both promote and inhibit inflammation (17). As COX-1 is expressed in nasal polyps as well, we postulate that elevated levels of COX-1 (and not COX-2) are responsible for the relative predominance of pro-inflammatory prostanoids in nasal polyposis. In this study we investigated the role of both enzymes in nasal polyp inflammation and studied the effect of topical GC treatment on nasal polyp inflammation.

Materials and methods

Subjects

NP ($n = 39$) were obtained from patients undergoing endoscopic sinus surgery (ESS). Prior to surgery a full ENT history was taken. Diagnosis of asthma was based on clinical features and when necessary on pulmonary function tests. Diagnosis of ASA intolerance was made on the basis of a clear-cut history of asthma attacks precipitated by nonsteroidal anti-inflammatory drugs (NSAIDs). Diagnosis of allergy was based on skin prick test (SPT) positivity. Presence or absence of treatment with topical and/or oral GC was recorded. Those patients treated with oral GC were excluded. Those patients ($n = 12$) treated with a topical GC spray (either fluticasone or budesonide 200–800 µg/day) for a period of at least 4 weeks prior to inclusion were included in the GC treated NP group. Those patients not treated with topical GC in the 4 weeks prior to inclusion were included in the non-GC treated NP group ($n = 27$) (Tables 1 and 2). Samples of healthy nasal mucosa ($n = 18$) were obtained from patients undergoing corrective surgery for turbinate hypertrophy or septoplasty. None of the patients in the control group had a

history of allergy, asthma, ASA intolerance, or GC treatment (Table 1).

Tissue handling

All specimens, obtained at the time of surgery, were immediately snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Immunohistochemical staining of CD68, CD117, MBP, elastase, IgE and COX-1

Six-micrometer thick serial sections of all snap-frozen specimens were cut on a Microm HM560 frigocut cryostat and transferred to amino-phosphate-ethylsilane (APES) coated slides (Starfrost, MarketLab, Caledonia, MI, USA), dried and stored at -70°C until analysis. Upon analysis, tissue sections were defrosted to room temperature, dried and fixed in acetone (cold as ice) for 10 min at room temperature. After fixation, tissue sections were rinsed with phosphate-buffered saline (PBS, pH 7.8), placed in a semi-automatic stainer (Shandon Sequenza, Waltham, MA, USA) and incubated with normal goat serum (CLB, Amsterdam, the Netherlands) for 10 min. Following the blocking procedure, sections were incubated with primary antibody for 60 min at room temperature. Mouse anti-human monoclonal antibodies directed against CD68, CD117, major basic protein (MBP), elastase, IgE and COX-1 were used (Table 3). All primary antibodies were diluted in PBS containing 1% (w/v) blocking reagent (10961760; Roche, Basel, Switzerland) to block endogenous avidin and biotin activity. Following incubation with primary antibody, sections were rinsed with PBS for 5 min and incubated with biotinylated goat anti-mouse antiserum (1 : 50, Biogenics Klinipath, Duiven, the Netherlands) for 30 min at room temperature. Next, sections were rinsed with PBS and incubated with streptavidin alkaline phosphatase (1 : 50, Biogenics Klinipath, Duiven, the Netherlands) for 30 min at room temperature. Sections were then rinsed with PBS containing tris(hydroxymethyl)aminomethane (TRIS) buffer (0.2 mol/l, pH 8.5) and incubated with new fuchsin (Chroma, Kongen, Germany) substrate (containing levamisole to block endogenous alkaline phosphatase (AP) enzyme activity) for

Table 1. Characteristics of the study population

	Normal mucosa ($n = 18$)	Nonsteroid treated NP ($n = 27$)	Steroid treated NP ($n = 12$)
Age (year), mean (SD)	31.6 (8.6)	45.6 (10.5)	48.1 (15.6)
Male, n (%)	14 (78.8)	14 (52)	5 (42)
Asthma, n (%)	0 (0)	13 (48)	6 (50)
ASA intolerance, n (%)	0 (0)	11 (41)	4 (33)
Allergy, n (%)	0 (0)	9 (33)	5 (42)

Table 2. Scheme of topical steroid spray used*

Dosage	Fluticasone	Budesonide
200 µg once daily	3	2
200 µg twice daily	1	5
400 µg twice daily	0	1

*Used for at least 1 month prior to biopsy ($n = 12$).

Table 3. Monoclonal antibodies and titers used

Antibody	Specificity	Cell type/cytokine	Titer	Source
KI-M6	CD68	Macrophages	1 : 200	DAKO (Glostrup, Denmark)
YB5.B8	CD117	Mastcells	1 : 65	Becton Dickinson (Alphen a/d Rijn, the Netherlands)
BB1	BB1	Basophils	1 : 150	A.F. Walls (South Hampton, UK)
MH25-1	IgE	IgE	1 : 750	CLB (Amsterdam, the Netherlands)
BMK-13	MBP	Eosinophils	1 : 100	Sanbio (Uden, the Netherlands)
AHN-10	Elastase	Neutrophils	1 : 3 20 000	Chemicon (Temecula, CA, USA)
IL-4 1-1	L-4	IL-4	1 : 150	Novartis (Basel, Switzerland)
IL-5	IL-5	IL-5	1 : 100	Tavernier (Ghent, Belgium)
IL-6	IL-6	IL-6	1 : 50	Sigma (Zwijndrecht, the Netherlands)
MH25-1	IgE	IgE	1 : 750	CLB (Amsterdam, the Netherlands)
COX-1	COX-1	COX-1	1 : 150	Sanvertech (Heerhugowaard, the Netherlands)
COX-2	COX-2	COX-2	1 : 50	Sanvertech (Heerhugowaard, the Netherlands)

20 min at room temperature. Sections were counterstained with Gill's hematoxylin, rinsed with distilled water and mounted in Vecta Mount (Vector, Burlingame, CA, USA). Control staining was performed by substituting primary antibody with PBS.

Tyramide signal amplification (TSA) staining for BB1, IL-4, IL-5, IL-6 and COX-2

A sensitive protocol was used based on the alkaline phosphatase method described above. Sections were cut and defrosted as described above. Sections were fixed in acetone (cold as ice) for 10 min, placed in the semi-automatic stainer and incubated with normal goat serum as described above. Slides were incubated with mouse anti-human monoclonal antibodies directed against BB1, IL-4, IL-5, IL-6 or COX-2 diluted in PBS containing 1% (w/v) blocking reagent (Table 3). After incubation with biotinylated goat anti-mouse antiserum, endogenous peroxidase was blocked using 0.2% (w/v) azide, 0.02% (v/v) hydrogen peroxide and 50% (v/v) methanol in PBS. Following the blocking procedure, slides were incubated with streptavidin conjugated peroxidase (NEN, Waltham, MA, USA) for 30 min at room temperature, rinsed with PBS and incubated with biotinyl tyramide in TRIS buffer for 10 min in order to amplify the signal. Next, slides were rinsed once again in PBS and incubated with alkaline phosphatase conjugated goat-anti-biotin antiserum (Sigma, Zwijndrecht, the Netherlands). Sections were then rinsed with PBS containing TRIS buffer (0.2 mol/l, pH 8.5) and incubated with new fuchsin (Chroma, Kongen, Germany) substrate (containing levamisole to block endogenous AP enzyme activity) for 20 min at room temperature. Sections were counterstained with Gill's hematoxylin, rinsed with distilled water and mounted in Vecta Mount (Vector, Burlingame, CA, USA). Control staining was performed by substituting primary antibody with PBS.

Light microscope evaluation

All sections were examined with an Olympus BX51 light microscope by two independent observers blinded to the experimental conditions. The number of positively stained cells were counted in the epithelium (per mm) and adjacent lamina propria (per mm²) at a final magnification of 200×. Results are expressed as the mean number of positive cells per mm or mm².

Data analysis

Statistical analysis was carried out by using spss 12.01 (SPSS Inc., Chicago, IL, USA). Data are expressed as median and interquartile range. Kruskal–Wallis nonparametric tests were performed to check for significant in between-group variability. In case of significant in between-group variability, Mann–Whitney-*U* nonparametric tests were performed for between-group comparisons. *P* values of less than 0.05 were considered statistically significant.

Results

Patient characteristics

No significant differences in patient characteristics were observed between non-GC treated and GC treated NP patients. Treatment regimens of those patients treated with topical GC are described in Table 2. The prevalence of asthma, allergy and ASA intolerance was similar in

both treatment arms (Table 1). None of the patients in the NM group suffered from asthma, allergy and/or ASA intolerance and none were treated with GC.

Normal nasal mucosa compared to non-GC treated nasal polyps

When comparing the presence of allergic mediators (IgE, IL-4) between NM and non-GC treated NP, it is clear that the number of IgE+ cells, but not IL-4+ cells, is significantly increased in NP epithelium and increased, although not significantly, in NP lamina propria. Compared to NM lamina propria, the number of cells positive for IL-5 and IL-6 (cytokines involved in eosinophil and neutrophil recruitment respectively) is increased in non-GC treated NP (Fig. 1). As one would expect, the number of eosinophils is markedly increased in both the epithelium and lamina propria of non-GC treated NP when compared to NM. For lamina propria, this increase is significant (Figs 1 and 2B). The number of neutrophils is slightly increased in non-GC treated NP lamina propria, but this increase is not significant. The number of mast cells is decreased in both epithelium and lamina propria of non-GC treated NP when compared to NM. For lamina propria, this decrease is significant. No differences are observed in the number of macrophages and basophils present. Compared to NM, the number of COX-1+ cells, but not COX-2+ cells, is significantly increased in non-GC-treated NP epithelium (Fig. 2A,B). No significant differences are observed in the number of COX-1+ and COX-2+ cells in NM and non-GC treated NP lamina propria (Tables 4A and 4B).

Non-GC treated nasal polyps compared to topical GC treated nasal polyps

When comparing GC-treated NP with non-GC-treated NP, no significant differences are observed in the number of IgE+ cells and IL-4+ cells in both NP epithelium and NP lamina propria. The number of IL-5+ cells, on the contrary, is significantly increased in GC treated NP lamina propria (Fig. 1). No differences are observed for NP epithelium. No differences are observed in the number of IL-6+ cells in both NP epithelium and NP lamina propria, although a tendency towards increased numbers of IL-6+ cells in GC treated NP lamina propria is observed. As one would expect, the level of tissue eosinophilia is decreased in both NP epithelium and NP lamina propria upon GC treatment, but this decrease is not significant (Figs 1 and 2B). A tendency towards increased numbers of neutrophils is observed upon GC treatment in both NP epithelium and NP lamina propria. As was shown for non-GC treated NP, the number of mast cells present in GC treated NP lamina propria is significantly decreased compared to NM and similar to levels present in non-GC treated NP lamina propria. No differences were observed in the number of macrophages and basophils present. As was described in the previous

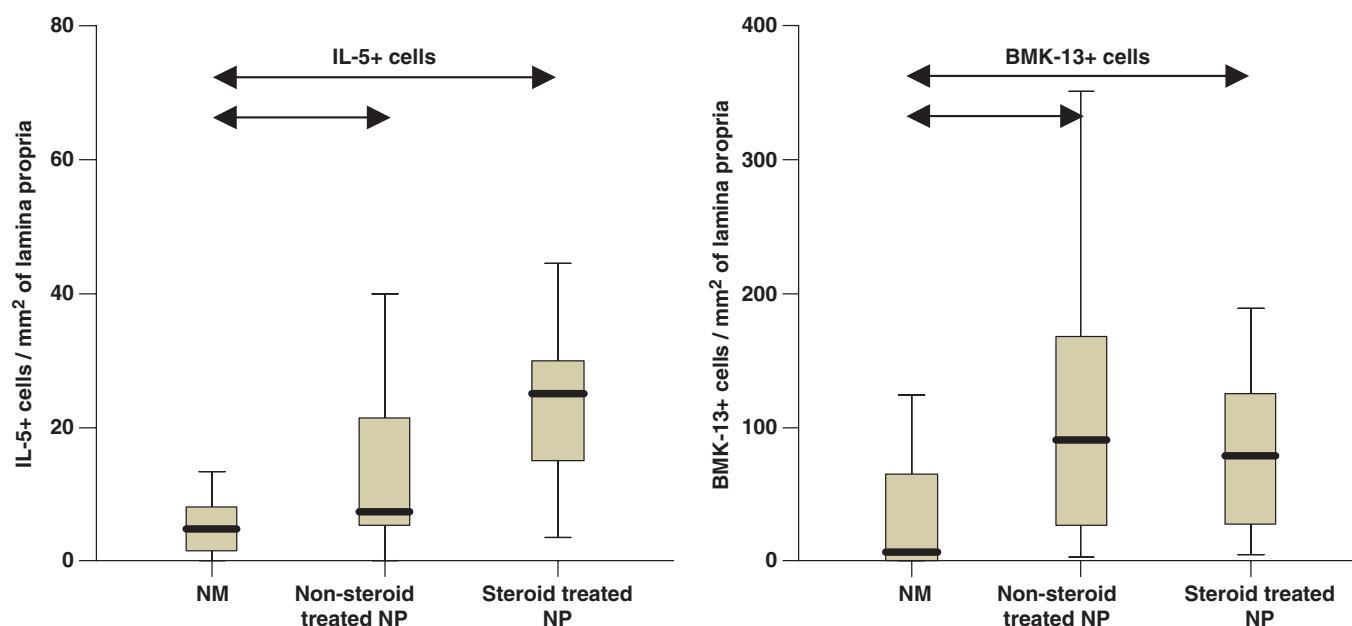


Figure 1. Influence of glucocorticoids on expression levels of eosinophils (BMK13) and interleukin 5 (IL-5) in the lamina propria of normal nasal mucosa and nasal polyps. *P* values < 0.05 are regarded as significant and are indicated by arrows.

section, the number of COX-1+ cells is significantly increased in non-GC treated NP when compared to NM. As is shown in Fig. 2A, treatment with GC results in a significant decrease in the level of COX-1+ cells in NP epithelium back to levels similar to NM. This striking difference between non-GC treated NP and GC treated NP is not observed for NP lamina propria. Compared to non-GC treated NP, a tendency toward increased levels of COX-2+ cells is observed for both NP epithelium and NP lamina propria of GC treated patients (Fig. 2A,B and Table 4A,B).

Discussion

Similar to findings by other groups, we demonstrate that NP are characterized by the presence of large amounts of eosinophils and increased numbers of IL-5+ cells and IgE+ cells (18, 19). Although other authors previously showed that treatment with oral GC resulted in a significant decrease in tissue eosinophilia and IL-5 protein levels (18), we show that treatment with topical GC does not result in a significant reduction in the number of eosinophils and IL-5+ cells in NP. Although tissue eosinophilia is reduced somewhat upon treatment with topical GC, the number of IL-5+ cells is not. Contrasting to what one would expect, the number of IL-5+ cells is instead increased significantly in topical GC treated NP. An explanation for this contradictory finding may be the fact that protein levels as assessed with enzyme-linked immunosorbent assay (ELISA) do not necessarily correlate with the number of positive cells as assessed with

immunohistochemistry (i.e. the total number of cells producing IL-5 may be increased, despite reduced IL-5 protein levels). As duration of topical GC treatment, type of drug formulation and dosage used varied among our included patients, this may also explain the lack of evidence to support the assumption that topical GC downregulate the number of eosinophils and IL-5+ cells in NP tissue specimens. As all NP were obtained from patients with persistent disease, we cannot exclude the possibility that some of our patients are resistant to topical GC. As was shown by Hamilos et al., fluticasone insensitive NP patients have a greater percentage of inflammatory cells expressing GC receptor β (GR β), a splice variant of the GR that is said to fail to bind GC. Hamilos et al. showed that in inflammatory cells expressing this GR β , the percentage of cells producing IL-5 is increased at baseline and is even further increased upon fluticasone treatment (20).

Compared to NM, the number of COX-1+ cells is significantly increased in non-GC-treated NP epithelium (Fig. 2A,B). Treatment with topical GC results in a significant reduction in the number of COX-1+ cells in NP epithelium back to the same level observed in NM epithelium. Although results are not significant, an opposite effect is observed for COX-2+ cells. The number of COX-2+ cells in non-GC treated NP is similar to NM, but is upregulated upon treatment with topical GC (Fig. 2A,B).

Several authors previously reported that GC downregulate COX-2 mRNA and protein levels *in vitro* (16, 21, 22). Consistent with previous findings from our group, in which we observed no *in vivo* effect of GC

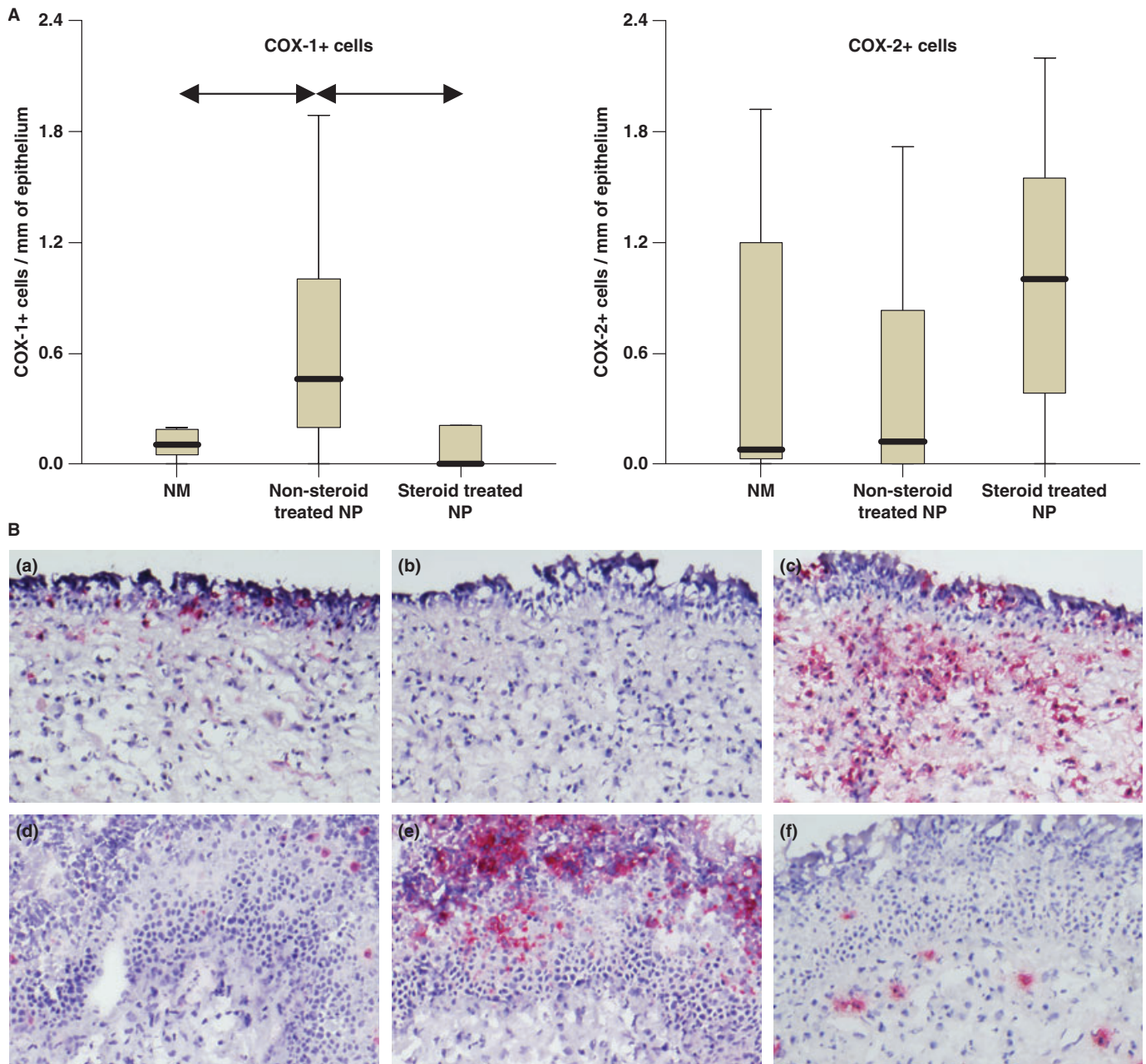


Figure 2. (A) Influence of glucocorticoids on expression levels of COX-1 and COX-2 in the epithelium of normal nasal mucosa and nasal polyps. *P* values < 0.05 are regarded as significant and are indicated by arrows. (B) Influence of glucocorticoids on the number of COX-1 positive cells, COX-2 positive cells and eosinophils. (a–c) A noncorticosteroid treated nasal polyp (magnification 20×). Note (a) the presence of increased numbers of COX-1 positive cells, (b) the presence low levels of COX-2 positive cells and (c) increased numbers of eosinophils (MBP) in nasal polyp epithelium and lamina propria. (d–f) A corticosteroid treated nasal polyp (magnification 20×). Note (d) the presence of low levels of COX-1 positive cells, (e) the presence increased numbers of COX-2 positive cells and (f) decreased numbers of eosinophils (MBP) in nasal polyp epithelium and lamina propria.

(topical and/or oral) on COX-2 mRNA expression (23), we do not show a significant effect of topical GC on COX-2 protein expression in NP cells *in vivo*. In the studies by Picado et al. (23) and Pujols et al. (24) we investigated the expression levels of COX-1 and COX-2 mRNA in NM and NP *in vivo*. COX-1 mRNA levels

were shown to be elevated in NP when compared to NM. Upon GC (topical and/or oral) treatment, a slight downregulation of COX-1 mRNA was observed (23). In our present study, the effect of topical GC on COX-1 and COX-2 protein expression levels was studied. In 2000 we reported that the effect of oral GC on COX-1

Table 4. Number of positive cells in (A) epithelium per mm and (B) lamina propria per mm²

	Normal mucosa (n = 18)	Nonsteroid treated NP (n = 27)	Steroid treated NP (n = 12)
(A)			
Macrophages (CD68)	2.3 (1.6–2.6)	1.3 (0.5–2.2)	1.7 (1.2–2.4)
Mast cells (CD117)	1.8 (0.3–3.8)	0.9 (0.0–3.1)	4.0 (0.12–6.1)
Eosinophils (BMK13)	0.0 (0.0–2.1)	1.0 (0.1–1.9)	0.0 (0.0–1.8)
Basophils (BB1)	0.0 (0.0–0.3)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
Neutrophils (Elastase)	3.1 (0.3–32.5)	1.7 (0.0–13.1)	9.3 (0.7–18.1)
IL-4	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
IL-5	0.0 (0.0–0.1)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
IL-6	0.0 (0.0–0.1)	0.0 (0.0–0.0)	0.0 (0.0–0.1)
IgE	0.0 (0.0–0.5)*	2.4 (0.4–9.1)*	2.5 (0.0–11.1)
COX-1	0.1 (0.1–0.2)*	0.5 (0.2–1.0)*‡	0.0 (0.0–0.4)‡
COX-2	0.1 (0.0–1.5)	0.1 (0.0–1.0)	1.0 (0.0–2.0)
(B)			
Macrophages (CD68)	107.5 (74.5–191.6)	115.9 (70.3–169.0)	146.0 (112.0–156.0)
Mast cells (CD117)	50.1 (32.4–67.5)*†	22.0 (15.1–44.0)*	31.2 (20.8–46.2)†
Eosinophils (BMK13)	6.6 (0.3–69.4)*†	90.8 (26.5–176.9)*	79.1 (23.9–131.8)†
Basophils (BB1)	2.4 (0.0–6.8)	1.8 (0.5–4.6)	1.7 (0.0–9.7)
Neutrophils (Elastase)	48.0 (22.3–163.9)	82.6 (26.9–149.7)	92.1 (16.7–185.2)
IL-4	5.0 (0.5–12.2)	1.0 (0.0–3.4)	1.7 (0.2–6.2)
IL-5	4.8 (1.3–8.8)*†	7.4 (4.7–22.3)*‡	25.0 (14.5–31.0)†‡
IL-6	0.0 (0.0–0.9)	0.5 (0.0–3.1)	1.2 (0.0–2.9)
IgE	24.3 (5.3–50.9)	31.6 (16.4–62.8)	62.2 (29.5–102.8)
COX-1	16.6 (13.7–26.5)	18.0 (12.6–26.3)	19.9 (17.8–26.1)
COX-2	0.5 (0.0–5.0)	0.4 (0.0–2.0)	1.0 (0.1–1.6)

Data are expressed as median (25th–75th percentile).

*Significant difference ($P < 0.05$) between normal nasal mucosa and nonsteroid treated NP.

†Significant difference ($P < 0.05$) between normal nasal mucosa and steroid treated NP.

‡Significant difference ($P < 0.05$) between nonsteroid treated NP and steroid treated NP.

mRNA expression levels in inflammatory nasal mucosa is only marginal (16). In our current study we demonstrate that topical GC (in contrast to oral GC) significantly downregulate COX-1 protein expression levels in nasal polyp cells *in vivo*. A possible explanation for this dichotomy, as suggested by Hoff et al. (21), is the lack of correlation between expression levels of COX-1 mRNA and expression levels of COX-1 protein. Post-transcriptional regulation may be one of the mechanisms that is involved in this process. A second explanation for the observed dichotomy may follow from observations by Kitzler et al., who showed that COX-1 mRNA exists in two splice variants: non-functional and functional COX-1 mRNA. Differentially or abnormally spliced, nonfunctional COX-1 mRNA may overestimate the level of functional COX-1 mRNA. As a consequence, the induction of functional COX-1 mRNA may be underestimated (22), thereby suggesting a lack of induction of COX-1 protein expression. Although the observed downregulation of COX-1 protein has been reported previously for GC treated cultured fetal pulmonary-artery endothelial cells and rheumatoid arthritis derived synovial cells (25, 26), we report for the first time that COX-1 protein levels are significantly downregulated in NP epithelium upon topical GC treatment.

In a previous study, we showed that nasal mucosa (including NP mucosa) expresses COX-2 mRNA rather constitutively (23, 24), data confirmed at the immuno-histochemical level by Gosepath et al. (27). Upon GC (topical and/or oral) treatment, we demonstrated a slight upregulation of COX-2 mRNA in NP tissue specimens (23). In our present study we show that COX-2 protein levels are also increased (although not significantly) in topical GC treated NP tissue specimens (Fig. 2A). This finding is in contrast with *in vitro* data by Aksoy et al. who showed a clear inhibitory effect of topical GC on COX-2 eicosanoid synthesis in cultured BEAS-2B airway epithelial cells (28). Based on our results, we conclude that one cannot predict the effect of topical GC on COX-2 protein expression levels *in vivo* based on the suppressability of COX-2 by topical GC *in vitro*. As it remains unclear whether data obtained from cultured airway epithelial cells can be extrapolated to NP epithelial cells, the result by Aksoy et al. should be interpreted with care. Having said this, in 1999, an interesting study by Gilroy et al. was published, showing that COX-2 may be pro-inflammatory during the early polymorphonuclear leukocyte-dominated phase (a.o. eosinophils), but may aid to the resolution of inflammation in the later, mononuclear-cell dominated phase (a.o. macrophages) by generating

an alternative set of anti-inflammatory prostaglandins, mainly prostaglandin E₂, in a carrageen-induced pleurisy model in rat (17). Thus, the relative upregulation of COX-2 upon topical GC treatment may skew prostaglandin production in NP towards a more anti-inflammatory profile and this, in turn, may result, together with the observed downregulation of upregulated NP epithelial COX-1, in the resolution of disease.

In summary, we report that topical GC significantly downregulate the number of COX-1+ cells and possibly upregulate the number of COX-2+ cells in NP epithelium. We postulate that differences in tissue GC concentration between oral and topical GC or binding

to different subsets of GC receptors account for this differential effect. Dexamethasone has been shown to increase COX-2 protein levels at low concentrations and to decrease COX-2 protein levels at higher concentrations in a mesangial cell culture system (29). Differences in GC receptor expression have been shown to account for the differential effects of dexamethasone on COX-2 expression in various cell types (30) and therefore it may well be that topical GC and oral GC stimulate different subsets of GC receptors, explaining the differential effect on cyclooxygenase (COX-1 and COX-2) expression. Future studies are necessary to confirm these hypotheses.

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