Original Article

Expression and localization of cyclooxigenases (Cox-1 and Cox-2) in nasal respiratory mucosa. Does Cox-2 play a key role in the immunology of nasal polyps?

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Abstract:
Introduction: Cyclooxygenases 1 (Cox-1) and 2 (Cox-2) play a key role in arachidonic acid metabolism and in the regulation of eicosanoid production. The balance of prostaglandin and leukotrien release in respiratory mucosa is a crucial factor in the development of Sampter`s triad in NSAID (aspirin-) intolerant patients and possibly also relevant in the pathophysiology and immunology of chronic rhinosinusitis (CRS) and nasal polyposis in NSAID tolerant patients. Methods: 36 surgical specimens were immunohistochemically labeled for Cox-1 and Cox-2. Specimens were taken from chronically inflamed mucosa (n=13) and from nasal polyps (n=10) during endonasal sinus surgery. Controls were obtained from healthy nasal respiratory mucosa (n=13), harvested during turbinate surgery in patients with nasal obstruction without inflammatory disease. Results: Analysis revealed that Cox-1 and Cox-2 were labeled in all 23 inflamed / polypoid tissue specimens and in all 13 controls. In chronically inflamed tissue the expression of Cox-1 and Cox-2 was strongly labeled in the respiratory epithelial lining and in mucosal glandular ducts. In nasal polyps the expression pattern of Cox-1 was similar, but Cox-2 was much less intensely labeled in the superficial epithelial cellular lining. Controls showed homogenious labeling of Cox-1 and Cox-2 in both tissues with little intensity. Conclusions: These data suggest that Cox-2 is downregulated in epithelial cells of nasal polyps. Cox-1 and 2 are present in high concentrations in ductal structures of mucosal glands. The significance of these findings has to be discussed with regard to the regulatory function of Cox-2 in eicosanoid release and the role of the latter in the immunology and pathophysiology of nasal polyps.

Key words: Cyclooxygenase, Cox-1, Cox-2, nasal polyps, immunology

Introduction

Several investigations have shown that arachidonic acid (AA) metabolism plays a key role in inflammatory reactions within nasal respiratory mucosa [1,2,3,4]. Cyclooxygenases (Cox-1 and Cox-2) catalyze the conversion of AA into prostanoids, leading to the formation of prostaglandin and thromboxane mediators. Prostaglandin E2 (PGE2), among others, has been described to have protective effects in contrast to other proinflammatory mediators of the eicosanoid pathway. Some prostaglandins have direct inhibitory effects on leukotrien production [5,6]. Cox exists in two isoforms with Cox-1 being the constitutive and Cox-2 being the inducible form. There is evidence that the expression pattern of both isoenzymes in respiratory mucosa is related to the pathophysiology of inflammatory disorders such as chronic rhinosinusitis (CRS) and / or nasal

polyposis and / or aspirin intolerance (AI). Reports by different authors have suggested that alterations of prostanoid metabolism might play a role in the ethiology of nasal polyps. However, several studies revealed conflicting findings on the up- or downregulation of both Cox-isoenzymes in inflammatory disease of respiratory mucosa [1,3,7,8,9,10].

In the present study immunohistochemical staining and labeling was used to separately identify Cox-1 and Cox-2 in tissue samples of surgical specimens. Specimens of chronically inflamed respiratory mucosa as well as specimens of nasal polyps were analyzed and compared to controls, which were specimens of healthy respiratory mucosa, harvested during septal and / or turbinate surgery. The aim of this investigation was to describe release patterns of the two isoenzymes in nasal respiratory epithelia under different mucosal conditions. Differences in the amount of enzymes released, their specific location within the respiratory mucosa as well as the role of these isoenzymes in the pathophysiology of CRS and / or nasal polyposis should be further elucidated.

Methods

Patients / Specimens

26 specimens of respiratory mucosa were harvested during sinonasal surgery. Thirteen specimens were obtained from patients with no history of chronic rhinosinusitis (8 male, 5 female, median age: 46 years), who underwent septal or turbinate surgery and / or rhinoplasty to improve nasal breathing. These 13 specimens were used as healthy controls. Twenty-three specimens were obtained during endonasal sinus surgery for either CRS without polypoid disease (n=13; 9 male, 4 female, median age: 42 years) or for nasal polyposis (n=10; 4 male, 6 female, median age: 48 years). Allergy testing revealed inhalant allergies in 5 of the CRS patients and in 4 of the 10 patients with nasal polyps. Four of the patients with nasal polyposis were diagnosed with aspirin intolerance.

Immunohistochemical staining / microscopic analysis

Tissues were snap frozen in liquid nitrogen immediately after resection and stored until further use at –80º C. For immunohistochemical staining (Table 1), slides were equilibrated to room temperature, fixed in ice cold acetone for 10 min, air-dried, and washed in TBS/Tween (0.05%) for 5 min. Endogenous peroxidase was inhibited by immersing slides in 3% H2O2/ Methanol for 20 min. Slides were washed in aqua dest and 5 min in TBS-Tween. After pre-incubation with 10 % normal serum in 1 % albumin bovine/PBS for 1 h to avoid unspecific binding, the primary antibodies were overlaid for 1 h at room temperature. Slides were washed 2 times with TBS-Tween, and consecutively incubated with biotinylated secondary antibody for 30 min, 2 x washed with TBS-Tween, incubated with streptavidine horse radish peroxidase-conjugate (1:200 in PBS; DAKO, Hamburg, FRG) for 30 min, washed again 2 x with TBS-Tween, and finally incubated with DAB/H2O2 (1.85 mM; Sigma, St. Louis, USA) for 1 min. Immediately after the staining development, slides were washed with aqua dest for 5 min and counter stained with hemalaun (1:5 in PBS; Merck, Darmstadt, FRG). Slides were rinsed for 5 min with aqua dest and dehydrated with isopropanol (80% -> 100%, each 10 sec.). Finally, samples were immersed in xylol (2 x 5 min.), closed with a cover slip and embedded with Enthelan (Merck, Darmstadt, FRG). Sections incubated without primary antibody served as negative controls. Sections of prostate tissue served as positive controls.

For evaluation of Cox-1 and –2 staining intensities and localization, three randomly chosen grids (25 µm X 25µm) at 400 X magnification were analyzed using an inverted microscope (Zeiss, Jena, FRG). Stainings were documented using PALM-Robosoftware, V. 1.2.3. (P.A.L.M. Microlaser technologies, Bernried, FRG) and

Table 1. Antibody concentrations

1st Antibody	Concentration	2nd Antibody	Concentration
Cox-1 (monoclonal mouse anti-human; Caymanm Ann Arbor, USA)	1:25/1:50	biotinylated goat anti-mouse (DAKO, Hamburg, FRG)	1: 250
Cox-2 (momoclonal rabbit anti-human; Assay Design, Ann Arbor, USA)	1:25/1:50	biotinylated goat anti-rabbit (DAKO, Hamburg, FRG)	1: 250

Dilutions of antibodies were prepared in 1% albumin bovine/PBS

images saved as jpg-files. Evaluation of all samples was performed by the same person to accomplish minimum variability.

Results

Controls (n=13)

In all 13 controls, Cox-1 and Cox-2 were equally labeled with low intensity in respiratory epithelial cells including their superficial cilia and in ductal structures of mucosal and submucosal glands. There was also some rather weak labeling within endothelial cells of vascular walls. (Figure 1 and Figure 2)

Chronically inflamed mucosa (n=13)

The 13 specimens of chronically inflamed mucosa without polypoid changes showed a markedly increased labeling of both isoenzymes in glandular ducts and in superficial epithelial cells. Compared to controls, both of these tissues were much more intensely labeled using both antibodies for Cox-1 as well as for Cox-2. No difference was observed between the concentration of these isoenzymes in ductal and epithelial tissues (Figure 3 and Figure 4).

Nasal polyps (n=10)

The labeling of Cox-1 was comparable to the pattern seen in chronically inflamed tissue. It showed identical intensities in ductal structures as well as in epithelial cells and cilia. However, Cox-2 revealed to be differently distributed in nasal polyps as opposed to Cox-1 on the one hand and to Cox-2 in chronically inflamed tissue on the other hand. It displayed a strong labeling in ductal glandular structures, but much less intensely within respiratory epithelia and cilia (Figure 5 and Figure 6). No difference was observed between samples of different patients in this group, regardless of whether or not patients had been diagnosed with inhalant allergies (n=4) and / or with aspirin intolerance (n=4).

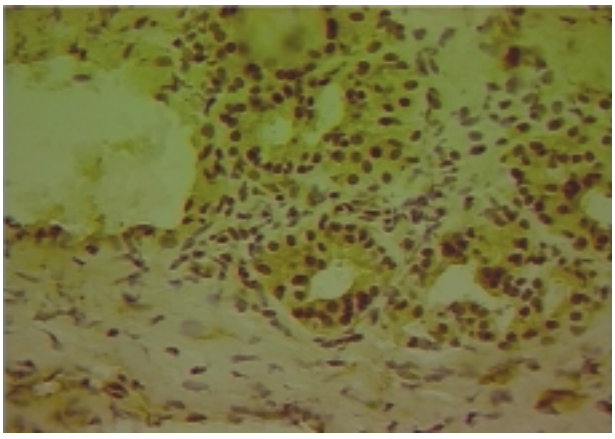
Discussion

It has been shown by several investigators that cyclooxigenases play a role in generating prostanoids [2,4] and that they might play a regulatory role in inflammatory disease of nasal mucosa, including nasal polyposis [1,9,11,12,13]. It has been widely agreed that Cox-1 is constitutively expressed, whereas Cox-2 is an inducible isoform [2,4,14]. It has been stated by different authors that an abnormal regulation of both Cox isoforms may be involved in the formation of nasal polyps [7,8,12]. However, there are conflicting reports as to whether it is a pure upregulation of Cox release, mainly involving Cox-2 [8,10,11,13] or if, on the contrary, it is a downregulation of Cox-2 release that is responsible for certain inflammatory changes, especially the formation of nasal polyps [1,7]. The heterogenity of these findings regarding up- and / or downregulation of Cox-2 may be partly due to the various pathophysiological factors of CRS and nasal polyposis, including inhalant allergies or aspirin intolerance, that are found in any patient population. Also differences in the methodology applied to evaluate the release of Cox-1 and Cox-2 (immunohistochemistry versus PCR) might have contributed to the nonhomogeneous findings described by different investigators [7,13,15]. Yun and colleagues [8], in accordance with other reports [9,11], described an upregulation of Cox-2 in nasal polyps. They concluded this from its labeling in submucosal glands and surface mucosa, while in healthy controls they found labeling of Cox-2 limited to the vascular wall [8]. The pattern described in nasal polyps is identical to our observation in the present study, however, we found both isoenzymes in these tissues in inflamed and healthy mucosa. Only in mucosa of nasal polyps we found a markedly weaker expression of Cox-2 in superficial epithelial cells. This finding corresponds to results of Pinto et al., who also applied immunohistochemistry to measure a ratio of Cox- and lipoxygenase (Lox) –release. The Cox / Lox ratio was decreased in nasal polyps compared to normal nasal mucosa [1]. Picado and colleagues described in 1999 that Cox-2 mRNA is downregulated in nasal polyps of aspirin-sensitive patients. Interestingly, PCR analysis did not reveal Cox-2 downregulation in nasal polyps from aspirin tolerant patients in this study [7].

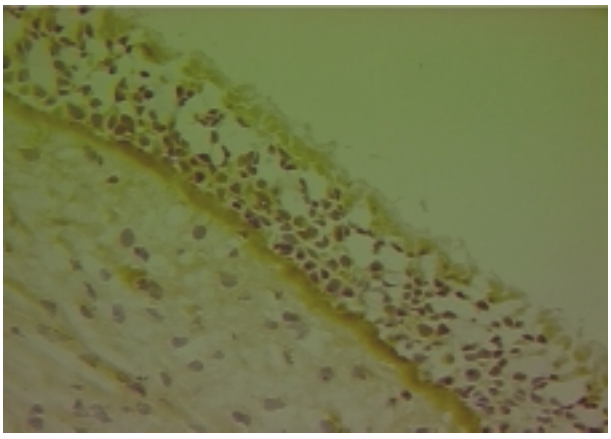
In contrast we found no difference between nasal polyps from patients with or without aspirin intolerance, although it should be kept in mind that only 4 of the 10 patients with nasal polyps were aspirin-sensitive. However, we clearly saw a downregulation of Cox-2 in superficial epithelial cells in all samples from nasal polyps. It could be hypothesized that this is related to a common pathway in the immunologic pathophysiology of nasal polyps. It is known that the inflamed tissue of nasal polyps generally contains large amounts of activated eosinophils [11]. Eosinophils are known to synthesize proinflammatory leukotriens that could play a role in initiating and /or maintaining the disease process in these patients. Interestingy, prostaglandin E2, which is one of the prostanoid mediators catalyzed by Cox-2, has a protective effect by reducing leukotrien synthesis in these cells [5,7]. In this scenario we hypothesize - in accordance in part with findings reported by Picado et al. for aspirin intolerant patients [7] - that a downregulation of Cox-2 might be related to polyp formation in general as it leads to a reduced PGE2 release and consecutively to a failure of this mediator to prevent excessive leukotrien production in inflammatory cells like eosinophils.

While Picado and colleagues used PCR analysis, other authors who used immunostaining also found no

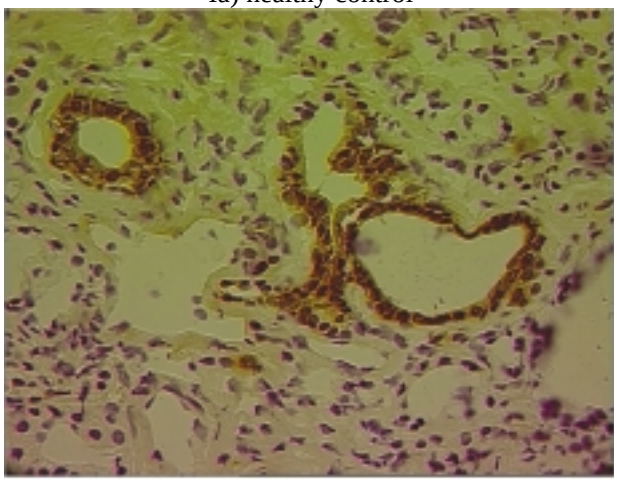
Glandular / vascular cells **Epithelial cells**



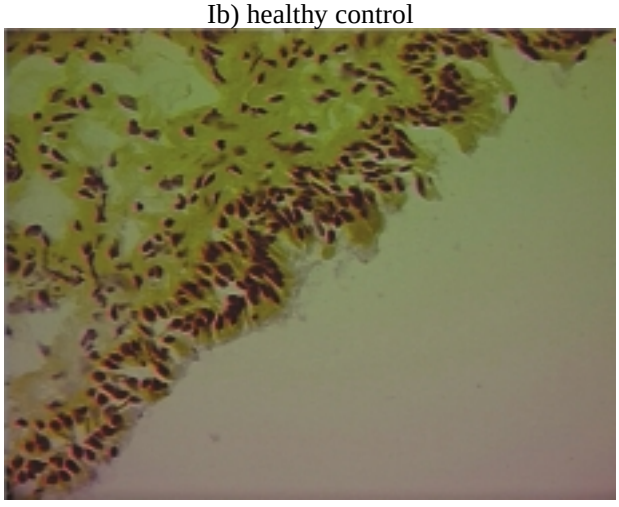
Ia) healthy control



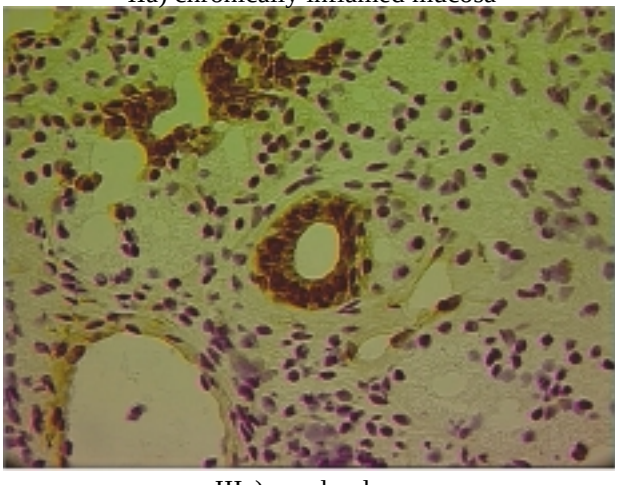
Ib) healthy control



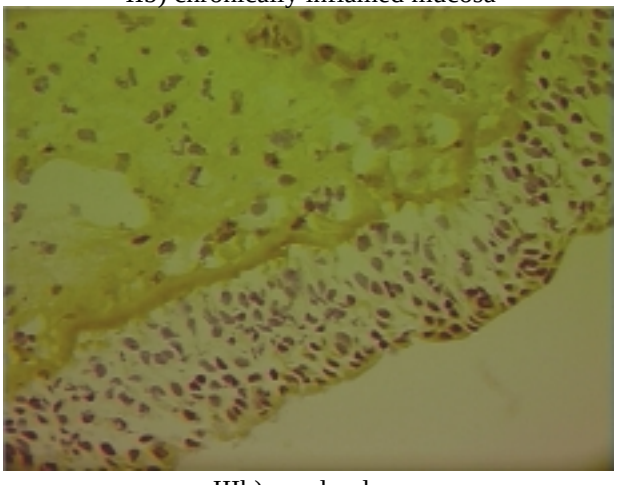
IIa) chronically inflamed mucosa



IIb) chronically inflamed mucosa



IIIa) nasal polyps



IIIb) nasal polyps

Figure 1: Cox - 2 labeling

I: Cox-2 low-intensity labeling in ductal structures of mucosal glands and vascular walls (a) as well as epithelial cells (b) in samples of healthy controls.

II. Cox-2 markedly increased intensity labeling in glandular ducts (a) as well as epithelial cells (b) in samples of chronically inflamed mucosa.

III. Cox-2 high-intensity labeling in glandular ducts (a), but of much lower intensity in epithelial cells (b) in samples of nasal polyps.

difference in the expression of Cox-2 in respiratory epithelia (bronchial mucosa) from aspirin-tolerant as opposed to aspirin-intolerant patients, confirming our present findings [13,15]. However, considering the small number of aspirin-intolerant patients within the group suffering from nasal polyposis, gradual differences between entities could possibly be unveiled in an analysis of larger patient groups.

Conclusion

Summarising, our results indicate that Cox-2 plays an important role in the modulation of inflammatory changes of nasal respiratory mucosa. Cox-1 and Cox-2 are strongly expressed in glandular structures and epithelial cells of chronically inflamed mucosa. In nasal polyps, however, Cox-2 is also expressed in glandular ducts, but downregulated in superficial epithelial cells. These findings suggest that formation of nasal polyps might be related to a decreased expression of Cox-2 in the respiratory epithelium. Further studies will be needed to unveil the exact immunologic cascade involved in this regulatory mechanism.

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