

Dynamics of COX-2 in nasal mucosa and nasal polyps from aspirin-tolerant and aspirin-intolerant patients with asthma

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Background: Only dynamic studies can elucidate the discrepancies concerning the expression of the inducible COX-2 gene in inflammatory airway diseases.

Objectives: To quantify the expression and spontaneous regulation of COX-1 and COX-2 mRNAs in nasal polyps and nasal mucosa by real-time PCR.

Methods: Nasal polyps were obtained from 16 aspirin-tolerant patients with asthma/rhinitis (ATAR) and 18 aspirin-intolerant patients with asthma/rhinitis (AIAR) undergoing nasal polypectomy. Nasal mucosa was obtained from 12 subjects undergoing nasal corrective surgery. All specimens were cut into 3 pieces. One was immediately snap-frozen in liquid nitrogen, and the remaining 2 were left at room temperature for 30 or 60 minutes before freezing. Data are presented as medians and 25th to 75th percentiles of 10⁶ cDNA molecules/μg total RNA.

Results: Baseline COX-2 mRNA levels were significantly lower in both ATAR (0.45; 0.13-1.20; $P < .05$) and AIAR (0.24; 0.12-0.41; $P < .001$) nasal polyps than in nasal mucosa (1.35; 0.52-3.90). COX-2 mRNA expression did not change over time in nasal mucosa but increased significantly in ATAR nasal polyps ($P < .05$), reaching similar levels to nasal mucosa after 60 minutes. In contrast, COX-2 mRNA showed no significant change over time in AIAR nasal polyps. COX-1 mRNA was higher in nasal polyps than in nasal mucosa, and its expression was not modified over time in any group of patients.

Conclusion: These results suggest differential kinetics of COX-2 mRNA between nasal mucosa and nasal polyps. AIAR nasal polyps appear to have a greater abnormality of the COX-2 pathway than ATAR. (*J Allergy Clin Immunol* 2004;114:814-9.)

Key words: Aspirin-induced asthma, aspirin-intolerant asthma/rhinitis, aspirin-tolerant asthma/rhinitis, rhinosinusitis, nasal polyps, COX, real-time PCR

Abbreviations used

AIAR: Aspirin-intolerant asthma/rhinitis

ATAR: Aspirin-tolerant asthma/rhinitis

NF: Nuclear factor

NSAID: Nonsteroidal anti-inflammatory drug

PGE₂: Prostaglandin E₂

UNG: Uracil-DNA Glycosylase

Aspirin-intolerant asthma is a clinical syndrome characterized by eosinophilic rhinosinusitis, nasal polyposis, aspirin sensitivity, and asthma.¹ There is an accumulation of evidence demonstrating low production of prostaglandin E₂ (PGE₂) in aspirin-intolerant patients with asthma/rhinitis (AIAR).²⁻⁶ Yamashita et al² and Schmid et al³ reported low levels of prostaglandins in nasal polyps from AIAR patients compared with those of aspirin-tolerant subjects with asthma/rhinitis (ATAR). Schäfer et al⁴ reported a significantly lower release of PGE₂ in peripheral blood cells from AIAR patients compared with ATAR subjects. Kowalski et al⁵ found that cultured epithelial cells obtained from the polyps of AIAR patients generated less PGE₂ than the cells obtained from ATAR patients. A similar abnormality in PGE₂ production by cultured bronchial fibroblasts of AIAR patients has recently been reported by Pierzchalska et al.⁶

Prostaglandins are generated through the COX pathway of arachidonic acid metabolism. Two isoforms of the COX enzyme are recognized: COX-1 and COX-2. COX-1 represents the constitutive isoform and performs a house-keeping function to synthesize prostaglandins that regulate normal cell activity.⁷ COX-2 is the inducible isoform, and its expression can increase in response to various stimuli, including cytokines and growth factors.⁷⁻¹⁰ Recently, new splice variants (COX-3, partial COX-1) derived from the *COX-1* gene have been characterized.¹¹ Their role in inflammatory responses is as yet unclear.

Asthma and chronic rhinosinusitis are inflammatory diseases of the airways that share common mechanisms.¹² Considering that upregulation of COX-2 is usually found in inflammatory diseases, the lowered production of PGE₂ in AIAR is an unexpected finding and suggests that the COX-2 pathway is abnormally regulated in these patients. In line with this hypothesis, we have reported that COX-2 is downexpressed in nasal polyps from AIAR patients.¹³ However, other groups have not found this abnormal regulation of COX-2 in bronchial biopsies, in which

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TABLE I. Characteristics of the study population

	Nasal mucosa	ATAR nasal polyps	AIAR nasal polyps
Subjects (n)	12	16	18
Sex (M/F)	10/2	11/5	4/14
Age (y)	42 ± 3	54 ± 4	50 ± 3
Bronchial asthma (yes/no)	0/12	8/8	18/0
Steroid treatment (yes/no)	2/10	8/8	11/7
Skin prick test (positive/negative)	4/8	6/8 ^a	4/12*

*The skin prick test could not be determined in 2 patients.

immunostaining for COX-2 showed no differences in AIAR patients compared with ATAR subjects.¹⁴

The expression of COX-2 is regulated by an immediate early gene whose transcription is increased in response to cell stimulation resulting from the activation of pre-existing, latent transcription factors, without requiring any intervening protein synthesis.¹⁵ As a result, COX-2 mRNA levels start to rise at approximately 30 minutes or sooner after stimulation and reach a maximal plateau within a few hours.^{8-10,16}

Differences in the methodology probably account for the reported discrepancies in the regulation of COX-2 in the upper and lower airways of AIAR patients. Because COX-2 is a rapidly inducible enzyme, differences in its expression in the airway mucosa should ideally be analyzed in dynamic kinetic studies rather than static conditions, which may provide only limited information on the role of an enzyme that is being continuously activated and deactivated.

We hypothesized that dynamic studies are the most adequate method for detecting differences in the regulation of COX-2. Because we have already demonstrated a delayed COX-2 mRNA production in nasal polyp explants exposed to either cell culture media or proinflammatory cytokines compared with healthy nasal mucosa,¹⁷ in the current study we were interested in analyzing the immediate early regulation of COX-2 mRNA in nasal tissues without the influence of any cytokine or other chemical stimulation. Thus, the objective of this study was to compare the early spontaneous regulation of COX-1 and COX-2 in nasal mucosa and nasal polyps from AIAR and ATAR patients.

METHODS

Subjects

Biopsies of nasal polyps were obtained from 16 ATAR and 18 AIAR patients. Nasal mucosa was obtained from 12 subjects undergoing nasal corrective surgery. The characteristics of the patients are summarized in Table I. The diagnosis of aspirin intolerance was made on the basis of a clear-cut history of asthma attacks precipitated by nonsteroidal anti-inflammatory drugs (NSAIDs). Aspirin sensitivity was tested by nasal challenge with lysine acetylsalicylic acid (L-ASA, 25 mg each nostril) only in asthmatic patients with an unknown or doubtful diagnosis, according to a method

TABLE II. Real-time PCR cycling conditions of the amplification program

Assay steps (segments)	Duration (s) COX-1/COX-2	Temperature (°C) COX-1 COX-2	
(I) Denaturation	10	95	95
(II) Primer annealing	6	60	57
(III) Elongation	40	72	72
(IV) Fluorescence acquisition	5	84	82

previously reported.¹⁸ The ATAR patients did not take any NSAID for at least 2 weeks before the biopsy extraction. The period between the last NSAID-induced asthma exacerbation in the AIAR patients and the collection of tissue varied from 4 months to several years. Of the ATAR patients with nasal polyps, 7 were on intranasal steroid therapy (fluticasone or budesonide, 400-800 µg/d), and 1 was receiving both fluticasone (400 µg/d) and oral prednisone (30 mg/d) at the time of surgery. Of the AIAR patients with nasal polyps, 4 were receiving intranasal steroids and 7 were receiving both intranasal and oral corticosteroids at the time of surgery. Within the nasal mucosa group, 1 patient was receiving intranasal fluticasone (200 µg/d) and 1 intranasal budesonide (800 µg/d). The nontreated patients had not received corticosteroids for at least 1 month before surgery.

None of the patients had upper respiratory infection during the 2 weeks before surgery. All subjects agreed to participate in the study, which was approved by the Ethics Committee of our institution.

RNA extraction and reverse transcription

Surgical biopsies from nasal polyps and nasal mucosa were cut into 3 pieces. One specimen was immediately snap-frozen in liquid nitrogen, and the remaining 2 were left at room temperature for 30 or 60 minutes before freezing. All tissues were kept at -80° C until analyzed. Total RNA from the nasal tissue specimens was isolated by using the RNeasy Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Total RNA was reverse-transcribed to cDNA by using random hexanucleotide primers and SuperScript II RNase H⁻ reverse transcriptase (Invitrogen, Paisley, United Kingdom), as previously reported.¹⁹

Primer design

COX-1 and COX-2 primers were designed to span introns.^{13,17,19} Their sequence was as follows: 5' TGCCAGCTCCTG-GCCCGCGCTT 3' (COX-1 sense, nucleotides: 516-539), 5' GTGCATCAACACAGGCGCTCTTC 3' (COX-1 antisense, nucleotides: 796-819), 5' TTCAATGAGATTGTGGGAAAAT-TGCT 3' (COX-2 sense, nucleotides: 574-600), 5' AGATCATCT-CTGCCTGAGTATCTT 3' (COX-2 antisense, nucleotides: 855-878). The resulting PCR products were of 304 bp (COX-1) and 305 bp (COX-2).

Generation of DNA external standards

After electrophoresis of COX-1 and COX-2 PCR products on a 2% agarose gel, the COX-1 and COX-2 bands were excised from the gel and were purified by using the QIAquick Gel Extraction kit (Qiagen). The COX-1 and COX-2 purified PCR products were immediately cloned into the pCR2.1 vector and transformed into competent *Escherichia coli* INVαF' cells (Invitrogen). Miniprepations of plasmid DNA (Qiagen) were performed for the selected colonies. To confirm the presence of insert, the isolated plasmids were digested with EcoRI and Hind III, and the digested products were resolved on an agarose gel. One clone containing either the

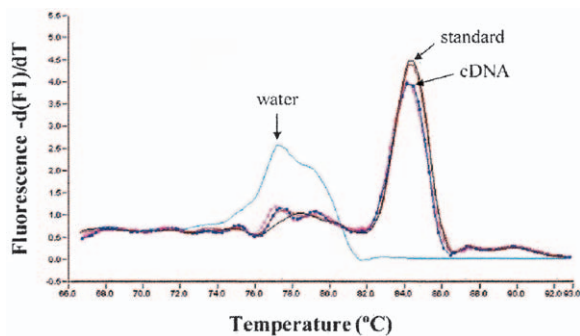


FIG 1. Melting curve analysis of COX-2 PCR products. The melting curves for the standard, sample cDNA, and primer-dimers (water) are shown.

COX-1 or COX-2 recombinant plasmid was selected and quantified through spectrophotometry. Dilutions of these plasmids were used as standards in each PCR run.

Real-time PCR

A master mix of the following reaction components (Roche Diagnostics, Mannheim, Germany) was prepared to the indicated end-concentration: 3 mmol/L $MgCl_2$, forward and reverse primers (0.5 μ mol/L for COX-1, 1 μ mol/L for COX-2); 2 μ L LightCycler Fast Start DNA Master SYBR Green I; 1 unit of heat-labile Uracil-DNA Glycosylase (UNG); and water, up to 18 μ L. Eighteen microliters master mix were filled in the glass capillaries, and 2 μ L reverse-transcribed total RNA (10 ng) or standard (plasmid double-stranded DNA) was added as PCR template. Capillaries were closed, centrifuged, and incubated at room temperature for 5 minutes to activate UNG. UNG eliminates PCR carry-over contaminations from previous DNA synthesis reactions. To improve SYBR Green I quantification, a high-temperature fluorescence measurement point was performed at each cycle (Table II, amplification program: segment IV). Such high temperature melts the unspecific PCR products, eg, primer-dimers; eliminates the nonspecific fluorescence signal; and ensures an accurate quantification of the desired product.

The following real-time PCR protocol was used: (1) a denaturation program (95°C for 10 minutes), which inactivates UNG and activates Fast Start DNA polymerase; (2) a 4-segment amplification and quantification program repeated 40 times, with a single fluorescence measurement at each cycle (Table II); (3) a melting curve program (65°C–95°C), with a heating rate of 0.05°C/s and continuous fluorescence measurements; and (4) a cooling program down to 40°C.

Statistical data analysis

Expression of COX-1 and COX-2 mRNA is given as median and 25th to 75th percentile of 10^6 cDNA molecules/ μ g total RNA. Nonparametric statistical analysis was performed by using the Friedman test and Wilcoxon rank test for within-group comparisons, and the Kruskal-Wallis test and Mann-Whitney *U* test for between-group comparisons. Spearman rank correlation was used when analyzing relationships between data. Statistical significance was set at $P < .05$.

RESULTS

Validation of the real-time PCR assay

Specificity of the COX-1 and COX-2 product was checked through gel electrophoresis and through melting

TABLE III. Characteristics and validation of the real-time PCR assay

Parameter	COX-1	COX-2
PCR product size (bp)	304	305
Limit of detection (molecules)	4	16
Quantification range (molecules)	12 to 4×10^5	48 to 1.6×10^6
Linearity (<i>r</i>)	0.99–1	0.99–1
PCR efficiency ($E = 10^{[-1/\text{slope}]}$)	1.8–2.1	1.8–2.1
Intra-assay variation (%*; <i>n</i> = 3)	3–10	3–10
Interassay variation (%*; <i>n</i> = 3)	8.9	7.3

*Expressed as variation of the number of molecules from the molecule number mean value (SD/mean).

curve analysis. The melting temperature was 86°C for COX-1 products and 84°C for COX-2, and primer-dimer formation was below 80°C (Fig 1).

The characteristics and validation of the real-time PCR assay are summarized in Table III. The sensitivity of the quantification, evaluated by using different starting amounts of the standard, was very high for both COX-1 and COX-2 PCR assays. Amplification efficiencies of target cDNAs differed from the standard in ≤ 0.05 . The assay precision was examined by analyzing 3 repeats of a given sample in the same run. The lowest was the starting amount of target mRNA, and the highest was the intra-assay variation. Interassay variation was analyzed by examining the same sample in 3 separate runs. Fig 2 shows a representative plot of logarithmic fluorescence versus cycle number for the COX-2 real-time PCR analysis provided by the LightCycler quantification software.

COX-2 mRNA regulation

We analyzed the expression of COX-2 mRNA in nasal polyp and nasal mucosa biopsies at baseline and after leaving the specimens at room temperature for 30 and 60 minutes before freezing. Baseline COX-2 mRNA levels were significantly lower in both ATAR (0.45; 0.13–1.20; *n* = 16; $P < .05$) and AIAR (0.24; 0.12–0.41; *n* = 18; $P < .001$) nasal polyps than in nasal mucosa (1.35; 0.52–3.90; *n* = 12; Fig 3). The lowest COX-2 mRNA baseline levels were found in AIAR nasal polyps, but these did not reach statistical significance with respect to ATAR nasal polyps.

The spontaneous expression of COX-2 mRNA did not change over time in nasal mucosa but significantly increased in ATAR nasal polyps ($P < .05$). In contrast, no significant increase in COX-2 mRNA expression was found over time in AIAR nasal polyps (Fig 3). Similarly to baseline findings, COX-2 mRNA levels after 30 minutes of biopsy extraction were significantly lower in nasal polyps (ATAR, $P < .001$; AIAR, $P < .001$) than in nasal mucosa. After 60 minutes, COX-2 mRNA expression was still lower in AIAR nasal polyps ($P < .01$) than in nasal mucosa, whereas COX-2 mRNA levels in ATAR nasal polyps reached those of nasal mucosa. After 60 minutes, COX-2 mRNA expression also became higher in ATAR ($P < .05$) compared with AIAR nasal polyps. It is worth

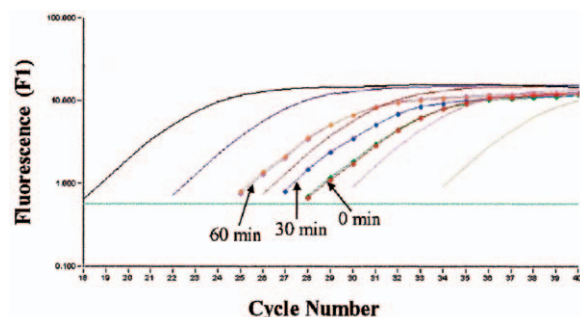


FIG 2. Quantification plot of COX-2 PCR. Serial dilutions of the standard and 2 replicates (dotted lines) of 3 samples (0 minutes, 30 minutes, 60 minutes) from a patient with aspirin-tolerant asthma are plotted.

noting the high variability of COX-2 mRNA values in nasal mucosa compared with that of nasal polyps. A remarkably low variability was found in COX-2 mRNA baseline levels in AIAR nasal polyps.

No differences were found in COX-2 mRNA expression, at baseline or in its regulation over time, between the ATAR patients with asthma and those without asthma. No association was found between COX-2 mRNA baseline levels and sex, age, or history of allergic rhinitis for any group of patients. COX-2 mRNA levels, either at baseline or after 30 and 60 minutes, did not significantly differ between patients who had or had not received corticosteroids before surgery.

COX-1 mRNA regulation

We also analyzed the expression of COX-1 mRNA in nasal polyp and nasal mucosa biopsies at baseline and after leaving the specimens at room temperature for 30 and 60 minutes before freezing. No significant differences in COX-1 mRNA expression were found at baseline between nasal mucosa and either ATAR or AIAR nasal polyps (Fig 4). However, when analyzing the ATAR and AIAR groups together, higher baseline COX-1 mRNA levels were found in nasal polyps (6.07; 2.84–11.3; $n = 34$; $P < .05$) compared with nasal mucosa (2.51; 2.11–4.52; $n = 12$).

COX-1 mRNA expression was not significantly altered over time either in nasal polyps or in nasal mucosa (Fig 4). Significantly higher COX-1 mRNA levels were found in nasal polyps after 30 minutes (ATAR, $P < .001$; AIAR, $P < .001$) and 60 minutes (ATAR, $P < .001$; AIAR, $P < .01$) of biopsy extraction compared with nasal mucosa. In contrast with COX-2 mRNA observations, very low variability was found in nasal mucosa COX-1 mRNA values, whereas a marked variability was found in nasal polyps from both ATAR and AIAR patients.

DISCUSSION

In this study, we have investigated the basal expression of COX-1 and COX-2 mRNAs and their spontaneous regulation over time in nasal polyp biopsies from both

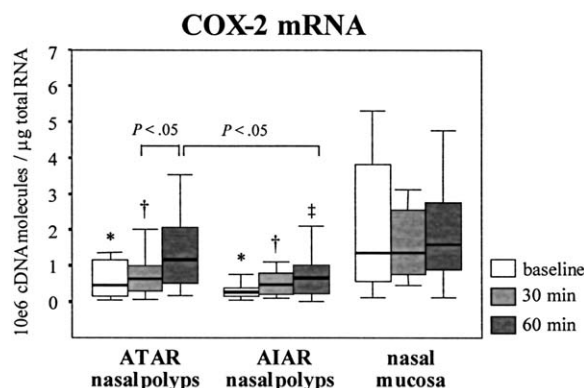


FIG 3. Spontaneous regulation of COX-2 mRNA in nasal polyps from ATAR and AIAR patients and nasal mucosa. Box plots show the 25th, 50th (median), and 75th percentile values. Whiskers show the minimum and maximum values. * $P < .05$, † $P < .001$, and ‡ $P < .01$ compared with nasal mucosa at equivalent time.

ATAR and AIAR patients and in nasal mucosa. To perform this study, we designed, optimized, and validated real-time PCR assays for COX-1 and COX-2. The amplification of the PCR products was linear over a wide range of input copies, with high sensitivity, precision, and reproducibility.

We have shown that the kinetics of the spontaneous COX-2 mRNA expression differ between nasal polyps and nasal mucosa. At baseline, there was significantly less COX-2 mRNA expression in nasal polyps, from both ATAR and AIAR patients, than in nasal mucosa. The spontaneous expression of COX-2 mRNA was not altered over a period of 60 minutes in nasal mucosa but increased significantly in ATAR nasal polyps. As opposed to ATAR, no significant increase in COX-2 mRNA expression was found over time in AIAR nasal polyps, a finding that suggests that the COX-2 gene has an impaired or delayed response in the nasal polyps of AIAR patients. It is noteworthy that the only stimulus involved in our study was the surgical resection of the tissue, and even in this situation, impaired COX-2 mRNA production was found in the AIAR patients, suggesting that these patients have an intrinsic alteration of the COX-2 pathway.

Our results are consistent with those of previous studies that reported a reduced production of PGE₂ in nasal polyps,^{2,3} peripheral blood cells,^{3,4} cultured nasal polyp epithelial cells,⁵ and bronchial fibroblasts⁶ of AIAR patients. However, this abnormality also appears to occur in ATAR, because a low production of PGE₂ has also been reported in bronchial fibroblasts⁶ and airway smooth muscle cells²⁰ from ATAR patients. The low production of PGE₂ in airway smooth muscle cells of these patients was accompanied by the simultaneous downregulation of COX-2 mRNA expression.²⁰ The low PGE₂ production detected in these studies also concurs with a recent study that shows that the concentration of PGE₂ in exhaled air is not increased in AIAR and ATAR patients with respect to control subjects.²¹ Therefore, in contrast with the generally accepted theory that COX-2 is upregulated, and thus

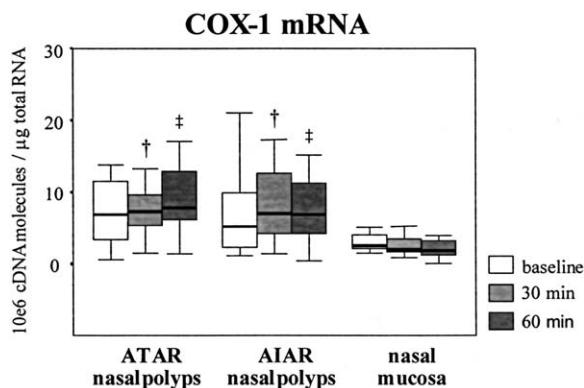


FIG 4. Spontaneous regulation of COX-1 mRNA in nasal polyps from ATAR and AIAR patients and nasal mucosa. Box plots show the 25th, 50th (median), and 75th percentile values. Whiskers show the minimum and maximum values. † $P < .001$ and ‡ $P < .01$ compared with nasal mucosa at equivalent time.

PGE₂ production is increased, in all inflammatory diseases,¹⁶ all of these findings suggest that the COX-2 pathway is not activated in the inflamed airways of patients with asthma and rhinosinusitis. The impaired capacity to upregulate the COX-2 pathway results in a diminished PGE₂ release. Because PGE₂ is known to have several anti-inflammatory actions in the human lung,²² a decrease in its production may contribute to the perpetuation of chronic inflammation and airway remodeling in patients with asthma and rhinosinusitis.

The reason the COX-2 pathway is not activated in asthma and nasal polyps is still unclear. The signaling cascade leading to COX-2 upregulation involves the participation of various transcription factors such as nuclear factor (NF)- κ B, NF-IL-6, and C/EBP β .⁷ We have recently reported a decreased activation of NF- κ B in nasal polyps of AIAR subjects,¹⁹ a finding that suggests that the impaired regulation of this transcriptional factor may account for the low expression of COX-2 in these patients.

The alterations in COX-2 mRNA expression that we have reported in nasal polyps¹⁷ and that Chambers et al²⁰ have reported in asthmatic airway smooth muscle cells contrast with results reported by other researchers in nasal and airway epithelium. Demoly et al²³ found COX-2 immunoreactivity in nasal epithelium of healthy subjects similar to that of patients with chronic sinusitis, nasal polyps, and chronic allergic rhinitis. The same group was also unable to detect any differences in COX-2 expression between the bronchial epithelium of normal control subjects and that of patients with asthma.²⁴ Conversely, other groups^{14,25,26} reported greater COX-2 immunoreactivity in the airways of patients with asthma compared with unaffected control subjects. Moreover, Pierzchalska et al⁶ did not find any differences in the expression of COX-2 mRNA in cultured bronchial fibroblasts of AIAR, ATAR, and control subjects, even though PGE₂ production was reduced in AIAR and ATAR fibroblasts after cytokine stimulation.

How can these discrepancies in COX-2 regulation be explained? A review of the studies reporting low PGE₂ production and downregulation of COX-2 in upper and lower airway cells and tissues of patients with asthma and/or rhinosinusitis reveals that almost all of them were dynamic studies in which cells and tissues were examined in time-course studies either spontaneously or after the addition of proinflammatory stimuli.^{3-6,17,20} Differences in COX-2 regulation and PGE₂ production were mainly detected over the course of the study rather than at baseline.^{6,17,20} In contrast, all studies reporting no differences or upregulation in COX-2 expression have used immunohistochemistry^{14,23-26} or RT-PCR⁶ at 1 time point, but no attempt has been made to assess the ability of the enzyme to adapt to situations under stress or inflammation.

In previous studies, we reported a significant downregulation of COX-2 mRNA in nasal polyps of AIAR with respect to ATAR nasal polyps.^{13,19} In the current study, the lowest COX-2 mRNA baseline levels were found in AIAR nasal polyps, but these did not reach statistical significance with respect to ATAR nasal polyps. However, COX-2 mRNA levels became significantly lower in AIAR compared with ATAR nasal polyps after 60 minutes. In view of the rapid induction of the COX-2 gene in ATAR nasal polyps, it is likely that differences in the time taken to handle and freeze the polyp specimen may account for the discrepancies in COX-2 mRNA expression that we and other authors have reported. Indeed, for a rapidly induced gene such as the COX-2 gene, the time at which biopsies are frozen after extraction is a key determinant in COX-2 gene expression studies.

We found higher COX-1 mRNA expression levels in nasal polyps than in nasal mucosa. In keeping with its housekeeping function,^{7,16} COX-1 mRNA showed very little change over time in both ATAR and AIAR nasal polyps and in nasal mucosa. It is worth noting the high variability in the COX-1 mRNA values of nasal polyps compared with those of nasal mucosa. This picture is the opposite of that shown for COX-2 mRNA, which was very variable in nasal mucosa but not in nasal polyps. A remarkably low variability was found in COX-2 mRNA baseline levels in AIAR nasal polyps. We are tempted to speculate that the high variability in COX-2 mRNA in nasal mucosa reflected the normal function of an adaptable gene in a healthy tissue, as opposed to an inflamed nasal tissue. Because both COX isozymes appear to function coordinately in inflammatory processes and it has been demonstrated that in certain physiological events, the lack of 1 COX isozyme can be compensated by the other,²⁷ we could speculate that the variability of COX-1 mRNA in nasal polyps may represent the intention of COX-1 to compensate—within its limited range of adaptability—for the incapacity of COX-2 to respond to a stressful situation.

In summary, COX-2 mRNA baseline levels were lower in both ATAR and AIAR nasal polyps than in nasal mucosa. We also demonstrated different kinetics of COX-2 mRNA between nasal mucosa and nasal polyps. The AIAR patients with nasal polyps appeared to have a greater abnormality of the COX-2 pathway than the ATAR

patients. The differential regulation of COX-2 mRNA over time between healthy and inflamed tissues highlights the importance of the tissue handling time for COX-2 gene expression studies. Finally, the diminished capacity of the airway cells of patients with asthma and nasal polyps to upregulate the COX-2 pathway, and the subsequent reduced release of PGE₂, could contribute to the perpetuation of chronic inflammation and airway remodeling in these patients.

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