Expression of Cyclooxygenase and Lipoxygenase Enzymes in Nasal Polyps of Aspirin-Sensitive and Aspirin-Tolerant Patients

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Objective: To evaluate the expression of cyclooxygenase (COX) and lipoxygenase (LO) enzymes in nasal polyp specimens from aspirin-sensitive (AS) and aspirin-tolerant (AT) patients.

Design: Immunohistochemical staining of archived tissue.

Subjects: Specimens from 26 patients (11 AS and 15 AT) with nasal polyps were analyzed; specimens from 4 patients were used as controls.

Interventions: Immunohistochemical techniques were used to evaluate the expression of the enzymes COX-1, COX-2, 5-LO, 12-LO, and 15-LO in nasal polyp tissue specimens from AS and AT patients. The results were compared with those of a control group of patients without a history of nasal polyposis or rhinosinusitis.

Results: Characteristic staining patterns of epithelium and submucosal glands were noted for each enzyme. Statistically significant (P<.05) differences in staining of columnar epithelium were noted for COX-1 (basal cell layer cytoplasm), COX-2 (apical cell layer cytoplasm), and 12-LO (full-thickness cytoplasm and nucleus). Increased 15-LO (full-thickness cytoplasm) expression in columnar epithelium was noted only in the AT group. Significant differences in the staining of submucosal glands were noted for COX-2 (plasma membrane and cytoplasm), 12-LO (cytoplasm), and 15-LO (cytoplasm) between control and AS patients as well as between control and AT patients (P<.05). The only significant difference noted between the AS and AT groups was cytoplasm staining for 5-LO in submucosal glands, which was greater in the AS group. No epithelial staining differences were noted between AT and AS patients.

Conclusions: There were significant differences in the expression of COX and LO enzymes between patients with nasal polyps and controls, irrespective of aspirin sensitivity. With 1 exception, there were no significant differences between AS and AT groups.


Approximately 10% to 15% of patients with nasal polyposis will have aspirin sensitivity and manifest the clinical condition known as the Samter triad. This condition includes aspirin intolerance, nasal polyposis, and asthma. Alterations in the arachidonic acid metabolism pathway have been implicated in the pathogenesis of nasal polyps, particularly in aspirin-sensitive (AS) patients. Prostaglandins (PGs) and leukotrienes (LTs) are the main products of the arachidonic acid pathway, and each appear to have wide-reaching influence on the development and regulation of inflammatory disease in the respiratory system. Prostaglandins are generated through the cyclooxygenase (COX) pathway of arachidonic acid metabolism, and LTs are synthesized through the lipoxygenase (LO) pathway. The COX pathway consists of 2 primary enzymes, ubiquitously expressed COX-1 and inducible COX-2. The LO pathway consists of 3 primary pathways: 5-LO, 12-LO, and 15-LO. There is accumulating evidence that low PG production combined with up-regulated LT synthesis may be important in the development of airway inflammation and subsequent sinonasal polyp formation. Recent studies have suggested that altered regulation of COX-1 and COX-2 may play an important role in the development of airway inflammation and sinonasal polyposis. The LTs, products of the LO pathway, are known to be important mediators in airway inflammation and overexpressed in many inflammatory respiratory conditions. However, our understanding of LO expression in sinonasal tissue remains extremely limited. It is not known if these changes in mediator levels are attributable to a qualita-
tive change in enzyme activity or to a quantitative difference in expression. Published reports differ regarding COX-1 and COX-2 expression in nasal polyps, with some authors reporting altered levels of protein or messenger RNA expression and others reporting no differences when compared with normal mucosa. Bronchial mucosal expression of COX-1 and COX-2 have been evaluated in patients with nasal polyps, with COX-2 found to be overexpressed relative to controls. Studies of bronchial mucosal specimens from patients with severe asthma have demonstrated increased expression of 5-LO and 15-LO. To our knowledge, no studies of LO expression in sinonasal mucosa have been reported for any patient population. The objective of this study was to use immunohistochemical techniques to evaluate the expression of COX (COX-1 and COX-2) and LO (5-LO, 12-LO, and 15-LO) pathway enzymes in nasal polyp tissue specimens from AS and AT patients.

METHODS

After approval was received from the Colorado Institutional Review Board of the University of Colorado at Denver and Health Sciences Center for the assessment of archived tissue, the surgical log of the principal surgeon (T.T.K.) was searched for patients who had undergone nasal polypectomy. A list of 26 nasal polyp specimens was thus produced. Each specimen was then assigned a nonidentifying letter to blind the evaluators. Four patients who underwent surgery for nasal obstruction uncomplicated by rhinosinusitis or endoscopic orbital decompression were also identified as a control group.

IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical reagents included murine COX-1 monoclonal antibody to sheep seminal vesicle COX-1, murine COX-2 monoclonal antibody to a polypeptide sequence from human COX-2, rabbit 5-LO polyclonal antibody to a peptide from human 5-LO, rabbit polyclonal antibody to murine 12-LO, and sheep 15-LO polyclonal antibody to rabbit reticulocyte 15-LO (Cayman Chemical, Ann Arbor, Mich). Five-micron tissue sections were mounted on charged glass slides (Superfrost Plus; Fisher Scientific, Pittsburgh, Pa) and baked overnight at 60°C. Deparaffinization with xylene and rehydration through a graded alcohol series was followed by blocking of endogenous peroxidase activity with 3% hydrogen peroxide for 15 minutes. Antigen retrieval was performed by heating in a decloaking chamber (Biocare Medical, Walnut Creek, Calif) in citrate buffer (20 mmol/L; pH, 6.0) at 120°C for 10 minutes. Staining was performed using an autostainer (DakoCytomation, Carpenteria, Calif). An indirect avidin-biotin immunoperoxidase method (Vector Laboratories, Burlingame, Calif) was used according to the manufacturer’s protocol for all cases except 15-LO, for which a biotinylated anti–sheep IgG (Sigma-Aldrich, St Louis, Mo) replaced the Vector IgG, which is specific for mouse and rabbit antibodies. For COX-1, ovarian carcinoma tissue was used as the control, and the final dilution was 5 µg/mL. For COX-2, prostate adenocarcinoma was used, with a final dilution of 1 µg/mL. Colon adenocarcinoma was used as the control for 5-LO and 15-LO, with final dilutions of 1:1900 and 1:250, respectively. The control tissue used for 12-LO was prostate adenocarcinoma, with a final antibody dilution of 1:1000. Paired serial sections were incubated at room temperature for 45 minutes with each particular antibody. Negative controls were performed on all sections using equivalent concentrations of subclass-matched IgG1 (Becton Dickinson Pharmigen, San Jose, Calif) generated against unrelated antigens for COX-1 and COX-2, rabbit serum for 5-LO and 12-LO, and anti–sheep IgG for 15-LO. Enzyme expression was visualized by development with 3,3′-diaminobenzidine (DakoCytomation), counterstained with hematoxylin, dehydrated in graded alcohols, and coverslipped.

BLOCKING STUDIES

To ensure the specificity of staining, blocking studies were performed when possible. Commercially available blocking peptides for the COX-1, COX-2, and 5-LO antibodies were used (Cayman Chemical). With a mole-mole excess of 100 for COX-1, 30 for COX-2, and 150 for 5-LO, the blocking peptide was incubated with the primary antibody for 45 minutes at ambient temperature. This step was followed by the standard staining protocol described above. No peptide or intact enzyme was available for blocking studies with 12-LO and 15-LO. Pancreatic tissue was used to perform a positive and negative control for 12-LO, as islet cells are noted to express the enzyme, while exocrine pancreas does not. This was confirmed using the standard concentration and staining protocol. No data regarding the tissue specificity for 15-LO expression were identified by a review of the literature.

GRADING

Each slide was evaluated by a pathologist (K.R.S.), and the staining intensity of each enzyme was scored on a scale of 0 to 3+ in the columnar epithelium and submucosal glands, based on a review of the entire histologic section. In cases with variable levels of staining, scores reflected the peak levels of staining. The median staining intensity scores were determined for each enzyme and were compared between the AS, AT, and control groups with the Mann-Whitney U test using a commercially available software package (SPSS Inc, Chicago, Ill).

RESULTS

DEMOGRAPHICS

A total of 30 specimens were evaluated. Four control specimens were harvested from patients without a history of rhinosinusitis or polyposis who underwent endonasal surgery for other indications (control group). Eleven specimens were collected from AS patients with documented asthma and nasal polyposis (AS group). Fifteen specimens were obtained from AT patients with nasal polyposis (AT group).

IMMUNOHISTOCHEMICAL ANALYSIS

Characteristic and reproducible staining patterns were observed for each enzyme, as shown in Figures 1, 2, 3, and 4. Cyclooxygenase-1 was localized to the cytoplasm of all epithelial and glandular cells that stained. Some stromal cells exhibited nuclear staining (Figure 1). Cyclooxygenase-2 was expressed in the plasma membrane and cytoplasm of stained epithelial
and glandular cells, with some nuclear staining noted in positive stromal cells (Figure 2). 5-Lipoxygenase was localized to the cytoplasm of epithelial and glandular cells, with 2 specimens demonstrating staining of epithelial cell plasma membrane (Figure 3). Among stained stromal cells, cytoplasm and nuclear staining for 5-LO were noted. 12-Lipoxygenase stained the cytoplasm and nucleus of epithelial, glandular, and stromal cells, with 1 specimen exhibiting plasma membrane staining of glandular cells (Figure 4). 15-Lipoxygenase staining was observed in the cytoplasm and nucleus of epithelial, glandular, and stromal cells that stained; 2 specimens displayed staining of epithelial plasma membrane (Figure 5).

**Cyclooxygenase-1**

Cyclooxygenase-1 expression in columnar epithelium was variably seen in all groups but, when present, exhibited nearly uniform cytoplasm staining of the full thickness of the epithelium. One AS specimen and 2 AT specimens demonstrated more intense staining in the basal cell layers of the epithelium. Staining was noted in 0 of 4 control specimens, 4 (44%) of 9 AS specimens, and 7 (41%) of 17 AT specimens. Strongly positive cytoplasm staining was noted in intraepithelial eosinophils in many speci-
mucosa epithelial expression (P = .01) and overall percentage of glands staining for COX-2 was significantly different than for the AS group (P = .02) as well as for the AT group (P = .002). Expression of COX-2 in the cytoplasm epithelial cells approached significant difference between the control group and the AT group (P = .06), while cytoplasmic expression between the control group and the AT group also approached significance (P = .07). No differences were noted between the AS group and the AT group (Table 2).

In submucosal glands, plasma membrane staining for COX-2 was noted in 3 (75%) of 4 control specimens, with cytoplasm staining observed in 1 specimen (25%) and no nuclear staining noted. In the AS group, plasma membrane and cytoplasm staining for COX-2 was noted in 8 (100%) of 8 specimens, with no nuclear staining observed. The difference in staining intensity of the plasma membrane (P = .03), cytoplasm (P = .007), and overall percentage of glands (P = .02) was statistically different between these groups. The AT group demonstrated plasma membrane staining in 12 (75%) of 16 specimens, cytoplasm staining in 15 (94%) of 16 specimens, and no nuclear staining. The differences in cytoplasm staining (P = .01) as well as the overall percentage of glands staining (P = .008) between the AT and control groups were statistically significant. There were no significant differences between the AS and AT groups (Table 2).

Cyclooxygenase-2

Columnar epithelium expression of COX-2 was commonly noted, with the apical one third of epithelial cells tending to stain more intensely than the basal cell layers. Of the control specimens, 1 (25%) of 4 displayed cytoplasm staining, while 0 showed plasma membrane staining. The AS group demonstrated cytoplasm staining in 8 (89%) of 9 specimens and plasma membrane staining in 5 (56%) of 9 specimens. No nuclear staining was noted. The AT group displayed cytoplasm and plasma membrane staining in 17 (100%) and 6 (35%) of 17 specimens, respectively. The percentage of columnar epithelial cells expressing COX-2 in control specimens was

5-Lipoxygenase

5-Lipoxygenase expression was also commonly noted in the columnar epithelium of all groups. Expression was confined to the cytoplasm, with the basal cell layers of the cytoplasm demonstrating more intense staining than

Table 1. Statistically Significant Staining Results for Cyclooxygenase-1

<table>
<thead>
<tr>
<th>Histologic Site</th>
<th>AS Group*</th>
<th>AT Group*</th>
<th>Control Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AS Group vs Control Group</td>
<td>AT Group vs Control Group</td>
<td>AS Group vs AT Group</td>
<td></td>
</tr>
<tr>
<td>Columnar epithelium</td>
<td>Cytoplasm staining score</td>
<td>1+ (1-3+)</td>
<td>1+ (0-3+)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Percentage of cells stained</td>
<td>5% (0-100)</td>
<td>1 (5-100)</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: AS, aspirin-sensitive; AT, aspirin-tolerant.
*Data are given as median (range).

Table 2. Statistically Significant Staining Results for Cyclooxygenase-2

<table>
<thead>
<tr>
<th>Histologic Site</th>
<th>AS Group*</th>
<th>AT Group*</th>
<th>Control Group</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AS Group vs Control Group</td>
<td>AT Group vs Control Group</td>
<td>AS Group vs AT Group</td>
<td></td>
</tr>
<tr>
<td>Columnar epithelium</td>
<td>Cytoplasm staining score</td>
<td>2+ (0-3+)</td>
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<td>Percentage of cells stained</td>
<td>100 (0-100)</td>
<td>50 (0-100)</td>
<td>0 (0-2)</td>
</tr>
<tr>
<td>Submucosal gland</td>
<td>Plasma membrane staining score</td>
<td>2+ (0-3+)</td>
<td>2+ (0-3+)</td>
<td>1+ (0-2+)</td>
</tr>
<tr>
<td></td>
<td>Cytoplasm staining score</td>
<td>2+ (1-3+)</td>
<td>1+ (0-3+)</td>
<td>0 (0-1+)</td>
</tr>
<tr>
<td></td>
<td>Percentage of glands stained</td>
<td>100 (0-100)</td>
<td>100 (0-100)</td>
<td>10 (0-80)</td>
</tr>
</tbody>
</table>

Abbreviations: AS, aspirin-sensitive; AT, aspirin-tolerant.
*Data are given as median (range).
the apical portion. Staining was noted in 100% of specimens in each group. No statistical differences were noted in overall expression, in expression in the apical cytoplasm, or in expression in the basal layer cytoplasm between groups (Table 3).

No submucosal gland plasma membrane staining was noted in any group. The control group demonstrated staining of the cytoplasm in 4 (100%) of 4 specimens and no nuclear staining. In the AS group, cytoplasm staining was observed in 8 (100%) of 8 specimens and nuclear staining was seen in 1 specimen (12%). No significant differences were noted between these groups. In the AT group, cytoplasm staining was noted in 12 (75%) of 16 specimens, with no nuclear staining. No significant differences were noted between the AT and control groups, but the difference in cytoplasm staining between the AS and AT groups was significant (P = .04). No other significant differences were noted between the AS and AT groups (Table 3).

12-Lipoxygenase

When present, staining for 12-LO was noted to be of uniform intensity through the full thickness of columnar epithelium, while 1 AT specimen demonstrated apical predominance. Staining for 12-LO was noted only in the cytoplasm of control specimens, with 2 (50%) of 4 displaying staining. No nuclear staining was seen in the control group. The AS group demonstrated cytoplasm staining in all specimens and nuclear staining in 8 (89%) of 9 specimens. Cytoplasm staining was noted in all AT specimens as well, with nuclear staining present in 12 (71%) of 17 specimens. When the control and AS groups were compared, the differences in overall staining (P = .03), cytoplasm staining (P = .006), and nuclear staining (P = .007) were statistically significant. When the control and AT groups were compared, the degree of overall staining (P = .005), cytoplasm staining (P = .01), and nuclear staining (P = .005) was significant. No significant differences were noted between the AT and AS groups (Table 4).

The control group had essentially no staining of submucosal glands for 12-LO, with the exception of 1 specimen that demonstrated cytoplasm staining of 5% of glands. The AS group demonstrated no plasma membrane staining, cytoplasm staining in 8 (100%) of 8 specimens, and nuclear staining in 6 (75%) of 8 specimens. When compared with the control group, the differences in cytoplasm staining (P = .03) and overall percentage of stained glands (P = .001) were significant. The AT group demonstrated plasma membrane staining in 1 (6%) of 16 specimens, cytoplasm staining in 16 (100%) of 16 specimens, and nuclear staining in 13 (81%) of 16 specimens. When compared with the control group, the differences in cytoplasm staining (P = .008) and overall percentage of stained glands (P = .001) were significant. No significant differences existed between the AT and AS groups (Table 4).

15-Lipoxygenase

Columnar epithelium staining for 15-LO also tended to be full thickness but with basal predominance. Plasma membrane staining was rare, noted in only 2 AT speci-

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**Table 3. Statistically Significant Staining Results for 5-Lipoxygenase**

<table>
<thead>
<tr>
<th>Histologic Site</th>
<th>AS Group*</th>
<th>AT Group*</th>
<th>Control Group*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submucosal gland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasm staining score</td>
<td>2+ (1-3+)</td>
<td>1+ (0-3+)</td>
<td>1+ (1-2+)</td>
<td>.29</td>
</tr>
</tbody>
</table>

Data are given as median (range).

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**Table 4. Statistically Significant Staining Results for 12-Lipoxygenase**

<table>
<thead>
<tr>
<th>Histologic Site</th>
<th>AS Group*</th>
<th>AT Group*</th>
<th>Control Group*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columnar epithelium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasm staining score</td>
<td>3+ (2-3+)</td>
<td>3+ (1-3+)</td>
<td>2+ (0-2+)</td>
<td>.006</td>
</tr>
<tr>
<td>Nuclear staining score</td>
<td>3+ (0-3+)</td>
<td>3+ (0-3+)</td>
<td>0</td>
<td>.007</td>
</tr>
<tr>
<td>Percentage of cells stained</td>
<td>100 (100)</td>
<td>100 (100)</td>
<td>100 (0-100)</td>
<td>.03</td>
</tr>
<tr>
<td>Submucosal gland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoplasm staining score</td>
<td>3+ (1-3+)</td>
<td>3+ (0-3+)</td>
<td>0 (0-2+)</td>
<td>.03</td>
</tr>
<tr>
<td>Nuclear staining score</td>
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<td>.11</td>
</tr>
<tr>
<td>Percentage of glands stained</td>
<td>100 (0-100)</td>
<td>100 (0-100)</td>
<td>0</td>
<td>.001</td>
</tr>
</tbody>
</table>

Abbreviations: AS, aspirin-sensitive; AT, aspirin-tolerant. *Data are given as median (range).
the difference in cytoplasm staining was significant ($P = .02$). In the AT group, no plasma membrane or nuclear staining was noted in any of the specimens, while cytoplasm staining was observed in 12 (75%) of 16 specimens. The difference in cytoplasm staining between the control and AT groups was significant ($P = .04$). There were no significant differences between the AT and AS groups (Table 5).

### Table 5. Statistically Significant Staining Results for 15-Lipoxygenase

<table>
<thead>
<tr>
<th>Histologic Site</th>
<th>AS Group*</th>
<th>AT Group*</th>
<th>Control Group*</th>
<th>AT Group vs Control Group</th>
<th>AS Group vs AT Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columnar epithelium</td>
<td>3+ (2-3+)</td>
<td>2+ (1-3+)</td>
<td>2+ (2-3+)</td>
<td>.14</td>
<td>.74</td>
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<tr>
<td>Cytoplasm staining score</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Submucosal gland</td>
<td>2+ (2-3+)</td>
<td>2+ (2-3+)</td>
<td>1+ (1-2+)</td>
<td>.02</td>
<td>.70</td>
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</table>

Abbreviations: AS, aspirin-sensitive; AT, aspirin-tolerant.
*Data are given as median (range).

In this study, we used immunohistochemical techniques to investigate the expression of COX-1, COX-2, 5-LO, 12-LO, and 15-LO in nasal polyp tissue. The data reported herein demonstrate significant differences in the expression of COX and LO enzymes between control and nasal polyp specimens. Very few differences were noted between the AS and AT patients, however. Our data are consistent with recent studies that have indicated that dysregulation of COX-1 and COX-2, and possibly the LO enzymes, may be important in the pathogenesis of polypoid rhinosinusitis.

The current study demonstrated increased expression of COX-1 in the epithelium in both the AS group and the AT group compared with the control group. No differences were noted between the AS and AT groups. The expression of COX-1 in submucosal glands was not significantly different between the groups either. Cyclooxygenase-1 is present in most tissues at baseline, and its expression is generally constitutive. Abnormal regulation of COX-1 has been implicated in the pathogenesis of nasal polyp formation in both AS and AT patients. Mullol et al. reported low expression of COX-1 in nasal polyp tissue obtained from AT patients without up-regulation after cytokine exposure. Pujols et al. reported somewhat conflicting data, with increased expression of COX-1 noted in both AT and AS tissue compared with controls, but again, there was no evidence of time-dependent up-regulation of expression. Recent work by Gosepath et al. using immunohistochemical techniques demonstrated increased COX-1 expression in both nasal polyp tissue and chronic inflamed sinonasal tissue without polyps compared with controls. In contrast, Demoly et al. found no difference in expression of COX-1 among control patients, patients with nasal polyps, and patients with sinusitis. Thus, our data combined with previous work appear to suggest dysregulation in COX-1 expression in patients with nasal polyposis. The exact mechanism of this abnormal regulation remains unclear and its importance to polyp formation ill defined.

Cyclooxygenase-2 is the inducible isof orm of the enzyme, and its expression can increase in response to a variety of stimuli, including cytokines and growth factors. It is partly responsible for the production of the PGs, which are believed to have both proinflammatory and anti-inflammatory functions. Our data demonstrated increased expression of COX-2 in both epithelial cells and submucosal glands in the AS and AT groups. Differences between the AS and AT groups again were not detected. Expression of COX-2 in nasal mucosa is up-regulated by proinflammatory cytokines such as interferon gamma, tumor necrosis factor alpha, and interleukin 1B, while this effect is blocked by dexamethasone. There is a growing body of work investigating COX-2 expression in sinonasal tissue in patients with nasal polyposis. The data from studies using similar immunohistochemical techniques have produced inconsistent results. Expression of COX-2 has been reported to be reduced in nasal polyp tissue compared with chronic nonpolypoid rhinosinusitis tissue. Demoly et al. reported no difference in the immunoreactivity of COX-2 in sinonasal epithelium of healthy controls compared with patients with rhinitis, sinonasal polyps, and chronic sinusitis. Additional work from the same group failed to demonstrate differences in COX-2 expression in bronchial tissue from normal controls compared with patients with asthma. In contrast, other investigators have reported findings similar to ours, noting increased...
immunoreactivity of COX-2 in patients with asthma compared with normal controls.11-13

Experimental methods may be important, however, when trying to understand these conflicting data. Recent work by Pujols et al1 using dynamic molecular techniques demonstrated differential kinetics of COX-2 messenger RNA between normal sinonasal mucosa and sinonasal polyp tissue in AT and AS patients. They demonstrated a down-regulation of COX-2, particularly in AS patients. Previous work by this same group of investigators demonstrated abnormal regulation of COX-2 expression in AT patients as well.7 These findings were recently supported by work from Perez-Novo et al.14 Thus, these data support the hypothesis that COX-2 dysregulation leading to decreased PGE2 production may be a critical factor in nasal polyp formation, in both AT and AS patients. Immunohistochemical techniques are limited in that only a single time point is evaluated, while dynamic methods assess the adaptive response of the enzyme. Dynamic methods could not be used in our investigation because archived tissue was studied. Nonetheless, our data are consistent with the overall concept that abnormal COX-2 regulation is important in the development of sinonasal polyposis. However, it remains unclear whether this is the result of variable enzyme expression and function or a consequence of posttranscriptional modifications to the enzymes. Further work in this area is required to understand these potential differences.

The LO pathway is the other “arm” of the arachidonic acid metabolic pathway and is responsible for the production of the leukotrienes. The enzyme 5-LO, together with 5-LO–activating protein, generates LT4 from arachidonic acid. Then, through the action of LTC4 synthase, the proinflammatory products LTC4, LTD4, and LTE4 are synthesized. Increased production of the LTs has been implicated in the pathogenesis of airway inflammation, including hyperplastic rhinosinusitis.15,16 LTC4 synthase is believed to be the rate-limiting enzyme in the production of LTs, and variable expression of this enzyme has been demonstrated in patients with polypoid sinusitis. The current literature investigating the role of the LO enzymes in airway disease is primarily limited to the lower airways.

In our study, all groups demonstrated expression of 5-LO, without differences between the study populations, with a single exception. Cytoplasmic expression of 5-LO was noted to be greater in the submucosal glands in the AS group compared with the AT group. The significance of this is unclear. Corrigan et al17 recently reported greater expression of the cysteinyl LT type 2 receptor in submucosal glands in sinonasal tissue obtained from AS patients compared with AT patients and controls. Therefore, differences in expression of 5-LO and the LO pathway in submucosal glands may be important, but further study is clearly required. Our data otherwise suggest that elevated 5-LO expression is not primarily responsible for increased LT production and may highlight the importance of posttranscriptional effects. It has been shown that the interaction between 5-LO and 5-LO–activating protein, a nuclear membrane protein, is a critical rate-limiting step in the activation of LT production by 5-LO.18 Therefore, the posttranscriptional effects of regulatory factors such as 5-LO–activating protein may be more important in the abnormal production of LTs than the absolute expression of 5-LO. Recent work has demonstrated increased 5-LO messenger RNA expression in nasal polyp tissue compared with controls, with some correlation to disease severity.14 Otherwise, the literature exploring the role of 5-LO expression in sinonasal disease is quite limited. There have been reports to suggest that use of the 5-LO inhibitor, zileuton, is beneficial in the management of nasal polyps and lower airway inflammatory diseases, thus indirectly suggesting that 5-LO activity may be an important factor in the pathogenesis of these conditions.19-21

The biologic role of 15-LO and its products remains poorly defined. It has been established that 15-LO is important in cell differentiation and maturation, inflammation, asthma, carcinogenesis, and atherogenesis. Initially, the products of 15-LO metabolism were believed to be proinflammatory; however, in recent years, several lines of experimental evidence have suggested that anti-inflammatory properties might be more important.22 According to this concept, the in vivo activity of 15-LO may be regarded as a protective response to limit inflammation. The main product of 15-LO metabolism of arachidonic acid is 15-hydroperoxyeicosatetraenoic acid (15-HETE), which has been associated with production of bronchial mucus, contraction of bronchial smooth muscle, and vascular permeability. Lipoxins, additional arachidonic acid metabolites of the 12-LO and 15-LO pathways, have been shown to be produced in nasal polyp tissue. These molecules serve as stop signals for leukocyte trafficking, facilitating the resolution of acute inflammation.23 Several studies have confirmed elevated expression of 15-LO in human lower airways, specifically in patients with asthma and atopic individuals.22 Perez-Novo et al14 found elevated 15-LO messenger RNA concentrations in tissue samples from patients with chronic rhinosinusitis and nasal polyps compared with controls; however, the degree of elevated expression was slightly lower in the AS patients with nasal polyps than in the AT patients with nasal polyps. Our study demonstrated increased epithelial expression of 15-LO in the AT patients compared with controls. We also noted increased cytoplasmic expression of 15-LO in epithelium and submucosal glands in both the AS group and the AT group compared with the control group.

The production of 15-HETE, an additional metabolite of 15-LO, has been noted to be increased in nasal polyp tissue, particularly from AS patients.24 When acetylated by aspirin, COX-2 is rendered incapable of producing PG products, but acquires 15-LO activity and produces 15-HETE. The precise role of 15-HETE in airway inflammation remains unclear but serves to highlight the complexity of these pathways. Thus, the differences in 15-LO expression may account for the differences in 15-HETE production noted in previous studies. It is also possible that these differences are attributable to production of 15-HETE by acetylated COX-2, particularly given the increased COX-2 expression noted in polyp specimens.
Our findings regarding 15-LO expression are consistent with those of Perez-Novoa et al., except that we were not able to demonstrate differences between our AS and AT groups. Both 15-HETE and lipoxins appear to be important products of 15-LO metabolism that have recently been linked to the presence of upper and lower airway inflammatory disease.14,23 These results support the hypothesis that 15-LO activity is important in the development of airway inflammation and deserves continued investigation.

The role of 12-LO and that of its major product, 12-HETE, are even less well defined in airway inflammation. A thorough search of Medline failed to identify previous reports of 12-LO expression in human sinonasal epithelium. We found increased expression of 12-LO in the epithelium and submucosal glands in both AS and AT patients compared with controls. Differences in expression between the AS and AT groups were not found. To the best of our knowledge, this is the first report of 12-LO expression in sinonasal epithelium. The biochemical and biologic properties of 12-LO and 15-LO appear to be similar, yet their tissue specificity and location differ. Expression of 12-LO has been well described in bovine airway epithelium, while 15-LO has been more extensively studied in human airway epithelium.23 The involvement of 12-LO expression in carcinogenesis, particularly metastasis, cellular apoptosis, and oxidative stress, has been previously reported.20-26 Studies have also shown that the Th2-derived cytokines up-regulate murine macrophage 12-LO expression in vivo and in vitro.30 Also, 12-HETE has been shown to induce COX-2 expression in pancreatic beta cells.31 Thus, the increased expression of 12-LO noted in our study may be in part responsible for the increased expression of COX-2 in our specimens. Induction of 12-LO by other products of the arachidonic acid pathway may exist and points to the complexity of this process. Despite intensive research on the expression and function of 12-LO in tissue systems other than respiratory epithelium, our understanding of the biologic significance of this enzyme in the airway inflammation is limited. Our data suggest an association between increased 12-LO expression and upper airway inflammation.

Several limitations of this study must be discussed. Because we were working with archived tissue, we were not able to use real-time polymerase chain reaction techniques as described by Pujols et al.1 Immunohistochemical staining techniques display enzyme expression at a single point in time, not allowing for evaluation of that enzyme’s ability to adapt. With select enzymes that appear to be inducible isoforms, such as COX-2, this difference in methodology may be important. Immunohistochemical techniques are important in defining the presence and location of the enzymes, and dynamic techniques help further elucidate the biologic properties of these select targets. Thus, the 2 types of techniques are complementary. An additional limitation of this study is the retrospective method in which the tissue and patients were identified. Corticosteroids are known to suppress the arachidonic metabolic pathway to varying degrees in various patient groups. We were not able to control for oral or nasal corticosteroid use in our study patients; therefore, the potential impact of these medications on our results is not clear. Our sample size is obviously small and may not be powered sufficiently. The information reported, however, appears important, particularly with regard to expression of the LO enzymes, which have not been previously studied in detail in sinonasal epithelium. Finally, it was not our intent to characterize the precise inflammatory cell populations that are responsible for enzyme expression in the study tissue.

In summary, we report differences in expression of COX and LO enzymes in nasal polytissue compared with controls. With the exception of increased 5-LO expression and a small sample size, we found no differences between the AS and AT groups. We describe the expression of 12-LO and 15-LO in sinonasal tissue and add to a very limited body of literature on this subject. Further investigation is necessary to determine the clinical significance of these differences and their relevance regarding potential therapeutic interventions targeted at the arachidonic acid pathway.

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REFERENCES


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**Trial Registration Required**

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