Glucocorticoid Receptor-β Overexpression According to Nasal Polyp Severity: Immunohistochemical Study

Yong-Hwi An, MD,1 Sung-Lyong Hong, MD,1 Doo Hee Han, MD,1 Jee Hye Wee, MD,1 Chae-Seo Rhee, MD1,2,3, Chul Hee Lee, MD1,2 and Yang-Gi Min, MD1,2
1Department of Otorhinolaryngology, Seoul National University Hospital; and 2Research Center for Sensory Organs; and 3Institute of Allergy and Clinical Immunology, Seoul National University Medical Research Center, Seoul, Korea

ABSTRACT

Background and Objectives: The aims of the study were to reintroduce a surgical technique for a “mini” Caldwell-Luc operation and to determine its efficacy.

Materials and Methods: A prospective study was performed in 23 patients undergoing the “mini” Caldwell-Luc operation. Improvements in clinical symptoms and endoscopic and computed tomographic (CT) findings were evaluated postoperatively over a follow-up period ranging from 6 to 21 months. All patients were surveyed for nasal symptoms (nasal obstruction, rhinorrhea, posterior nasal drip, headache, and anosmia), complications (tooth or gum pain, numbness, persistent facial pain, and facial hypo-paresthesia), and recurrence. Preoperative nasal polyps were classified by the Gaskins method, and preoperative paranasal sinusitis was graded according to the Kennedy CT staging system.

Results: Symptom scores were all significantly reduced postoperatively (paired t-test, p < 0.05). There were no major complications specific to this technique. Among 29 maxillary sinuses that received the “mini” Caldwell-Luc operation, seven showed recurrence (24%).

Conclusion: The “mini” Caldwell-Luc operation provides an alternative method of obtaining access to the maxillary antrum and is associated with minimal morbidity.

KEY WORDS: Caldwell-Luc operation · Chronic maxillary sinusitis · Endoscopic sinus surgery.

INTRODUCTION

Nasal polyposis which is characterized by edematous inflamed mucosa is a chronic inflammatory disease of the nose and paranasal sinuses.1 Although glucocorticoids have been widely used for the treatment of nasal polyp, responses to corticosteroid therapy are frequently unsatisfactory. Recent studies regarding glucocorticoid receptor (GR) have demonstrated its important role in the cellular regulation against glucocorticoid resistance.2-4 This study is based on the assumption that the mechanisms of steroid insensitivity in human tissues are related to the change in receptor distribution.

Two subclasses of GRs have been identified: GR-α which performs normal functions, and GR-β which structurally does not bind to glucocorticoid and, therefore, functionally related to steroid insensitivity.3 The pathogenesis of steroid dependency and resistance is thought to be a complex network of many different factors though there is no general agreement on these responses. Focused on a molecular basis of corticosteroid receptors in human nasal tissues, an up-regulation of GR-β expression has been reported to be a landmark of steroid resistance in nasal polyps.6

In this study, we used immunohistochemistry to evaluate the expression of GRs in steroid-insensitive nasal polyps and compared it with that in normal nasal mucosa. We also determined whether the expression of GR-β in nasal polyps would differ according to their severity.

MATERIALS AND METHODS

Patients

Nasal biopsy specimens including the nasal polyp and
middle turbinate mucosae were obtained from 16 patients diagnosed with chronic rhinosinusitis and nasal polyps between September 2006 and September 2007 (9 males, 7 females; mean±SEM, 48±12 years) who underwent endoscopic sinus surgery (the nasal polyp group). These patients did not have a history of asthma, aspirin sensitivity or allergic rhinitis. In this study, oral or intranasal corticosteroids were administered in all patients. Eleven patients were treated with topical corticosteroids for at least 4 weeks. Five patients were treated with systemic glucocorticoids for at least 2 weeks. None of the nasal polyps responded to steroid therapy. Systemic and intranasal steroids were discontinued for minimum 1 month before biopsy. Samples of the middle turbinate tissue from 4 patients (2 males, 2 females; mean±SEM, 42±15 years) who underwent septorhinoplasty or transphenoidal hypophysectomy were used as controls (the control group). These 4 patients had no history of airway diseases including sinonasal disease. All patients in the nasal polyp and control groups gave written informed consent to the study. This study was approved by the Institutional Review Board of Seoul National University Hospital (H-1008-049-326).

Staging of nasal polyps

The classification system described by Lund and Mackey for grading nasal polyposis was used: grade 0, no visible polyp; grade 1, mild polyp(s) confined to middle meatus; grade 2, moderate polyp(s) beyond middle meatus but not completely obstructing the nasal cavity; and grade 3, severe polyp(s) completely obstructing the nasal cavity. Endoscopic grading of nasal polyps was recorded by a single observer the day before operation.

Immunohistochemistry

Biopsy samples from the polyps and normal nasal mucosae were processed for immunohistochemical staining using the avidin-biotin complex method as previously described. GR-α specific polyclonal rabbit antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a dilution of 1:200, and rabbit GR-β polyclonal antibody (Calbiochem, San Diego, CA) at a dilution of 1:100 were prepared as primary antibodies. Biotinylated anti-rabbit peroxidase secondary antibody (Vector Laboratories, Burlingame, CA) was used at a dilution of 1:200 to detect these primary antibodies. The brown immunoperoxidase reaction was developed with diaminobenzidine, and the sections were counterstained with hematoxylin. For negative control preparations, primary antibodies were replaced with nonspecific rabbit immunoglobulin.

Quantification

Positive cells for each of the primary antibodies were counted through use of a light microscope (Leitz microscope, Wetzlar, Germany) with an eyepiece reticle at x200 magnification. To avoid the observer bias, tissue sections were assessed by a single investigator in a blind fashion. The 0.2-mm² reticle was oriented beneath the epithelial basement membrane, and both positive and negative cells were counted at 5 randomly selected fields.

Statistics

Data were expressed as mean±SEM. Between-group comparisons were performed by using the Mann-Whitney U test. The relationship between the expression of GR-β and the severity of nasal polyps was determined by the Wilcoxon signed rank test. Differences were considered statistically significant at p<.05.

RESULTS

Grading of nasal polyp

Of the total 16 patients, 1 had mild polyps (grade 1 nasal polyp), 10 had moderate polyps (grade 2 nasal polyp) and 5 had severe polyps (grade 3 nasal polyp). The control group had no polyp (Table 1).

Distribution of GRs in nasal tissues

Immunostaining was positive for GRs in the nasal polyp and control groups as illustrated in Fig. 1. Receptor-positive cells were stained brown and were predominantly localized in the epithelial cells and submucosal

Table 1. Demographics and endoscopic grading of nasal polyp

<table>
<thead>
<tr>
<th></th>
<th>Nasal polyp group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male : female)</td>
<td>9 : 7</td>
<td>2 : 2</td>
</tr>
<tr>
<td>Median age (year)</td>
<td>48</td>
<td>42</td>
</tr>
<tr>
<td>NP grade*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No NP</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Mild NP</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Moderate NP</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Severe NP</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

NP : nasal polyp. * : According to the Lund and Mackey staging system by endoscopic findings
inflammatory cells. There were more cells expressing GRs in the epithelium than in the submucosa of the nasal polyp and control groups.

**Comparison of GR-α expression**

GR-α-positive cells were found in both nasal polyps (53 ± 15 cells/mm²) and middle turbinate mucosae (51 ± 8 cells/mm²) from the nasal polyp group as well as middle turbinate mucosae (43 ± 11 cells/mm²) from the control group. The absolute numbers of these cells in nasal polyps from the nasal polyp group were not significantly different from those in the control group (p > .05). The absolute numbers of these cells in nasal polyps from the middle turbinate from the nasal polyp group were not significantly different from those in the control group (p > .05) (Fig. 2).

**Comparison of GR-β expression**

GR-β was expressed in the nasal polyps (36 ± 8 cells/mm²) and middle turbinate mucosae (25 ± 4 cells/mm²) from the nasal polyp group and the middle turbinate mucosae (17 ± 3 cells/mm²) from the control group. GR-β was more expressed in nasal polyps from the nasal polyp group than in middle turbinate mucosae from the control group (p = .01). In addition, the number of GR-β-positive cells was significantly increased in the middle turbinate mucosae from the nasal polyp group as compared to the middle turbinate mucosae from the control group (p = .03) (Fig. 3).

**Association of GR-β expression with the severity of nasal polyps**

For grade 2 nasal polyps, immunohistochemical stain-
We hypothesized that GR-β may be expressed in nasal polyps, and recent studies have shown that GR-β overexpression in topical steroid-insensitive nasal polyps is associated with steroid unresponsiveness. In this study, we investigated the expression of GR-β in nasal polyps and compared it to normal nasal mucosa.

The expression of GR-β was determined in grade 2 and 3 nasal polyps. In grade 2 polyps, GR-β was positive in 57 ± 9 cells/mm², whereas in grade 3 polyps, the expression was statistically higher at 60 ± 18 cells/mm². These results are similar to those of previous studies with multiple human tissues and are consistent with the findings of earlier research. Expression of GR-β in the epithelium may not have clinical implications because most types of inflammatory cells such as eosinophils and T lymphocytes are predominant in the submucosa. However, our results indicate that the epithelium has a crucial role in the interactions between insensitivity to glucocorticoid and polyp development. There is no doubt that the submucosa is the main site of the inflammatory process in polyp formation.

We demonstrated in this study that the number of GR-β-positive cells was increased in nasal polyps. In contrast, there was no evidence of overexpression of GR-α in nasal polyps compared to normal mucosa. The ratio of GR-β-positive to GR-α-positive cells representing corticosteroid unresponsiveness has been shown to differ among previous studies. Our results revealed that the ratio of GR-β-positive to GR-α-positive cells was higher in grade 3 nasal polyps than in grade 2 nasal polyps. Furthermore, the GR-β/GR-α ratio tended to increase in steroid-resistant nasal polyps compared to normal turbinate mucosa, but this difference was not statistically significant (p=0.058, data not shown). Although there is still controversy regarding GR-β function, we assumed that up-regulation of GR-β is a marker of steroid insensitivity for nasal polyps. GR-β was more expressed in severe nasal polyps than in moderate polyps. This potentially explains the molecular basis of unresponsiveness to glucocorticoid therapy in nasal polyps. Such possibility may be supported by in vitro superantigen-induced GR-β expression in a nasal explant model and by in vivo GR-β overexpression in topical steroid-insensitive nasal polyps.

The small number of specimens is a limiting factor in this study. In contrast to many studies on nasal polyps, in which samples of nasal polyps were used as study groups, we think that using both nasal polyps and middle turbinate mucosa will give a more accurate representation, as the majority of nasal polyps arise from middle turbinate. This choice, however, limited the number of specimens, as removal of middle turbinate mucosa is not often performed in patients with nasal polyps. Non-specific immunostaining of inflammatory cells is also a weak point of our study. Some studies showed the details of immunoreactivity for inflammatory cells such as T lymphocytes, eosinophils, and macrophages, but others didn’t. To understand the significance of the location of GR-β expression in nasal polyps, a detailed anal-
sis of cell types should be continued.

CONCLUSION

We demonstrated that GR-β is more-expressed according to nasal polyp severity by using the immunohistochemical staining. The results of this study suggest that increased expression of GR-β in nasal tissue may contribute to the pathogenesis and progression of nasal polyps and that the relationship between the expression of GR-β and the severity of nasal polyps may be related to the progression of nasal polypositis and steroid resistance. Further studies are needed to confirm our results.

Acknowledgments

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REFERENCES