Gene Expression Changes of Inflammatory Mediators in Posterior Laryngitis Due to LPR Reflux and Evolution with PPI Treatment: A Preliminary Study

Susan Thibeault, PhD, University of Wisconsin – Madison
Marshall Smith, MD University of Utah
Kathryn Peterson, MD, University of Utah
Ritta Vittalo-Moller, MD, Ph.D, Karolinska University

Supported by a grant from Eisai, Inc and PriCara, Unit of OrthoMcNeil, Inc.

Introduction

- LPR has an estimated occurrence in 10% of patients visits to Otolaryngologists
- Clinical manifestations attributed to inflammation of the mucosa
- In esophagus, elevated levels of chemokines/cytokines have been measured in GERD with decreased levels measured after treatment with PPI indicating that constitutive inflammation responds to drug management.
- Little is known regarding chemokines/cytokines profiles in LPR and after treatment with PPI.

Aims of Study

1. Measure molecular gene expression levels of commons and well recognized mediators of inflammation, from mucosal biopsies of the PL in persons with LPR
2. Measure gene expression levels in these mediators from mucosal biopsies of the LP in person LPR after a 10 week trial with rabeprazole (20 mg BID)
   - Interleukin 6 (IL6)
   - Interleukin 8 (IL8)
   - Interleukin 1a (IL1a)
   - Interleukin 1b (IL1b)
   - Transforming growth factor beta one (TGFb1)
   - Vascular endothelial growth factor (VEGF)
   - Fibroblast growth factor 2 (FGF2)
   - Tumor necrosis factor alpha (TNFα)

25 Adult Participants

- No history of H&N malignancy, gastroesophageal or laryngeal injury or surgery, chronic sinusitis or rhinitis or smoking within past year

Videoystroboscopy
- Rigid exam
- Laryngoscopic signs consistent with LPR (Vaezi et al)
- Consensus agreement between 2 judges

Esophageal manometry and 24 hour pH monitoring
- If taking PPI must have discontinued for 10 days prior to pH study
- Dual probe pH monitoring - proximal sensor 1 cm below LES, distal sensor 15 cm below that
- Avoid pH data (percent time pH<4) from the proximal electrode were analyzed separate for the total, upright, and supine positions
- Decrease in pH below 4 not induced by eating or drinking was considered the beginning of a reflux episode and the following rise to pH above 5 was considered the end
25 Adult Participants
No history of H&N malignancy, gastroesophageal or laryngeal injury or surgery, chronic sinusitis or rhinitis or smoking within past year

Transnasal Laryngeal Biopsy
- Biopsy taken before and after treatment
- Biopsy from the interarytenoid area in the PL

Treatment
- 10 week 20 mg bid dose of rabeprazole
- Instructed to take 30 minutes before meals
- No other medications for HB or LPR taken
- Compliance was measured by pill count at second biopsy
- Considered adequate if 75% or more of the medication was taken
- At second biopsy asked to rate their improvement in their symptoms using a 10 point Likert scale

Methods
- RNA isolation
- Reverse transcriptase
- Real time polymerase chain reaction

Results
- 42 participants eligible for 24 hour monitoring
  - 3 could not tolerate probe
  - 10 normal results
  - 4 withdrew after 1st biopsy
  - 25 participants
    - 15 no history of taking PPI
    - 10 history of taking PPI
    - No significant differences in initial gene expression levels for any of the mediators as assessed by Wilcoxin Rank Sum

Compliance 93% (range 85-100)
- Overall 6.96 ± 1.02
- No previous history of PPI 6.7 ± 0.95
- History of PPI 7.12 (± 1.06)
Table 2: Significant correlations between 24 hour pH parameters (proximal esophagus) and pretreatment gene expression levels for all patients, those without history of PPI usage and those with history of PPI usage. Correlation Coefficient (p value)

<table>
<thead>
<tr>
<th>Gene</th>
<th>All Participants</th>
<th>Participants with NO PPI History</th>
<th>Participants with PPI History</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-1</td>
<td>-0.447 (0.04)</td>
<td>-0.740 (0.006)</td>
<td>-0.596 (0.04)</td>
</tr>
<tr>
<td>VEGF</td>
<td>-0.471 (0.03)</td>
<td>-0.699 (0.01)</td>
<td>-0.596 (0.04)</td>
</tr>
<tr>
<td>IL1a</td>
<td>-0.457 (0.03)</td>
<td>-0.629 (0.02)</td>
<td>-0.471 (0.03)</td>
</tr>
<tr>
<td>IL1b</td>
<td>-0.459 (0.03)</td>
<td>-0.699 (0.01)</td>
<td>-0.471 (0.03)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>-0.520 (0.01)</td>
<td>-0.681 (0.01)</td>
<td>-0.520 (0.01)</td>
</tr>
<tr>
<td>FGF-2</td>
<td>-0.605 (0.003)</td>
<td>-0.734 (0.006)</td>
<td>-0.605 (0.003)</td>
</tr>
<tr>
<td>IL6</td>
<td>-0.497 (0.02)</td>
<td>-0.683 (0.02)</td>
<td>-0.497 (0.02)</td>
</tr>
<tr>
<td>IL8</td>
<td>-0.471 (0.03)</td>
<td>-0.693 (0.02)</td>
<td>-0.471 (0.03)</td>
</tr>
</tbody>
</table>

Spearman Correlation Analysis
Table 3: Linear regression analysis (p values) for genes and medication group, compliance, number of abnormal events and improvement scores.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Premedication</th>
<th>Compliance</th>
<th>Improvement</th>
<th>Number of abnormal acid events in the proximal esophagus</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFβ1</td>
<td>0.0396</td>
<td>0.4144</td>
<td>0.6456</td>
<td>0.3524</td>
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<tr>
<td>VEGF</td>
<td>0.0216</td>
<td>0.2585</td>
<td>0.3131</td>
<td>0.0235</td>
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<tr>
<td>IL1α</td>
<td>0.5791</td>
<td>0.6128</td>
<td>0.5945</td>
<td>0.6662</td>
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<tr>
<td>IL1β</td>
<td>0.2022</td>
<td>0.5086</td>
<td>0.9874</td>
<td>0.5765</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.3413</td>
<td>0.4996</td>
<td>0.8059</td>
<td>0.1924</td>
</tr>
<tr>
<td>FGF2</td>
<td>0.4944</td>
<td>0.5401</td>
<td>0.4060</td>
<td>0.9149</td>
</tr>
<tr>
<td>IL6</td>
<td>0.1045</td>
<td>0.7544</td>
<td>0.4157</td>
<td>0.3724</td>
</tr>
<tr>
<td>IL8</td>
<td>0.0237</td>
<td>0.4911</td>
<td>0.1914</td>
<td>0.2126</td>
</tr>
</tbody>
</table>

Discussion

- Evidence for differing phenotype of the disease
- Those with previous history of PPI had greater levels of cytokines after treatment suggesting greater inflammation
- May represent phenotype that gets worse despite PPI treatment
- May also indicate disease progression, such that differing noxious refluxate may be contributing to their inflammation

Lack of significant change in gene expression levels of cytokine mediators after a 10 week treatment indicates little therapeutic change in laryngeal tissue response to PPI

This is consistent with placebo controlled trials that have demonstrated limited or no benefit from PPI for treatment of LPR

Different that findings in the esophagus

Perhaps duration was insufficient

Belafsky et al report symptoms resolution after 8 weeks without physical findings were slower to resolve

Site of biopsy -- Hill et al report no change in posterior cricoid commissure hypertrophy after 30 months of acid suppression therapy

Negative correlations were measures for those participants with no history of PPI usage

Most prevalent in the supine position

Typically would expect to find positive correlation in the upright position
Limitations

- Only 25 participants
- Not controlled
- Based enrolment upon stroboscopy finding which has been documented to be indiscriminate at best
- Sensitivity of pH monitoring in detecting acid reflux with the proximal probe has been reported to be 55% or less
- No post biopsy assessment

Conclusions

- First study to measure and report gene expression level of inflammatory cytokine and chemokines medication in the larynx
- Provide cellular evidence of differing phenotypes of the disease

Acknowledgments

- Kristen Thomas, UU, Study Coordinator
- Cara Sauder and Kristine Tanner, UU, Subject Recruitment
- Wenhua Li, UU, Molecular Analysis
- Glen Leverson, UW Madison, Statistical Assistance