

Proton-Pump Inhibitor Treatment in Eosinophilic Esophagitis is Associated with Decreased Eosinophil Degranulation

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Abstract

Eosinophilic esophagitis (EoE) is an increasingly recognized disorder whose diagnosis is based on histologic demonstration of eosinophilic inflammation. We previously reported improvement of symptoms in children with EoE on long-term proton-pump inhibitor (PPI) monotherapy despite persistent eosinophilic inflammation. We sought to determine whether PPI monotherapy in children with EoE is associated with decreased eosinophil degranulation. Ten children with EoE had esophageal biopsies assessed for eosinophil, mast cell and Langerhans cell concentration and eosinophil degranulation at diagnosis and following PPI treatment. There was no significant difference in cell concentrations between initial and one-year follow-up. A significant decrease in number of free lying granules was observed at the one-year follow-up biopsies. Prolonged PPI therapy in EoE is associated with decreased eosinophil degranulation.

Keywords: Eosinophilic esophagitis; Proton-pump inhibitors; Degranulation

Introduction

Eosinophilic esophagitis (EoE) is an increasingly recognized disorder. Diagnosis of EoE is based on histologic demonstration of eosinophilic inflammation, with ≥ 15 eosinophils per high power field (hpf) being the cutoff most commonly used, in the absence of pathologic reflux as evidenced by a normal pH monitoring study or persistent inflammation on high-dose proton pump inhibitor (PPI) treatment [1]. Symptoms include feeding issues, vomiting, chest or abdominal pain, dysphagia and food impaction [2].

Eosinophils contain multiple toxic granules, whose content include basic proteins, cytokines, chemokines, lipid mediators, and oxygen radicals. Free-lying granules serve as a surrogate marker for released eosinophil products that promote tissue damage, inflammation, remodeling, and fibrosis [3]. Eotaxin-3 is a chemokine stimulated by T-helper cytokines to recruit and activate eosinophils. PPI's have been shown to block eotaxin-3 release, suggesting a role independent of acid production [4]. We previously reported improvement of symptoms in children with EoE on long-term PPI monotherapy despite persistent eosinophilic inflammation [5]. We hypothesized that symptomatic improvement in our patients was due to a PPI effect in eotaxin 3-induced degranulation. Therefore, we sought to determine whether the improvement with PPI monotherapy was associated with decreased eosinophil degranulation.

Methods

We reviewed the records and biopsies from ten patients previously described [5] with EoE who improved clinically on PPI monotherapy. All patients were between 1 month and 18 years old, were on an unrestricted diet, were diagnosed with EoE based on standard criteria [1] with persistence of eosinophils on follow-up biopsies 3 months

after diagnosis while on PPI, and had undergone follow-up endoscopy at least one year after diagnosis.

Hematoxylin and eosin-stained sections were reproduced from formalin fixed paraffin-embedded, endoscopically-obtained esophageal biopsies and evaluated to quantify eosinophils and assess degranulation. Immunohistochemical staining with tryptase for mast cells, and S100 for Langerhans cells using standard methodology was performed to evaluate their presence and possible role in eosinophil recruitment.

Using 400X magnification, all intraepithelial eosinophils were counted in three separate fields that had the highest density of eosinophils in each slide. The average number of eosinophils for these three fields was reported. The same method was used to quantify mast cells (tryptase) and Langerhans cells (S-100).

Using 600X magnification, areas with the highest concentration of scattered eosinophil granules were photographed using a Nikon Digital Sight DS-Fi1 microscope camera. Each image was printed on standard short bond paper (21.5 cm x 28 cm, representing a microscopic field of 7752 μm^2). A 5.3 cm x 6.8 cm rectangular frame (corresponding to a microscopic area of 1938 μm^2) was used to designate the areas with the highest density of free-lying eosinophil granules. All free-lying granules within this framed area in three separate fields were counted, and the mean was reported.

Statistics

Comparison of results between presentation and most recent endoscopy was performed using paired t-test. Results are expressed as mean \pm SD.

This study was reviewed and approved by the Institutional Review Board of the North Shore - LIJ Health System.

Results

Demographics

The mean age of the patients was 6.6 ± 4.8 years with 7 males. Presenting complaints were dysphagia (4), failure to thrive (3), abdominal pain (1) and vomiting (2). The patients had a second follow-up biopsy 17.1 ± 8.5 months following initiation of PPI. The mean PPI dose was 1.5 ± 0.6 mg/kg. Nine patients were treated with lansoprazole and one child with esomeprazole. All patients were either asymptomatic (n=9) or significantly improved (n=1) on PPI monotherapy.

Comparison between biopsies at diagnosis and follow-up on PPI therapy

There was no significant difference in eosinophil count between initial and follow-up endoscopies. Compared to the initial endoscopy, the granule count decreased by the 3-month follow-up endoscopy and significantly decreased by the 1-year follow-up (Table 1).

	Mean \pm SD	p value*
Eosinophils/hpf		
Initial endoscopy	106.2 \pm 38.6	
3-month endoscopy	76.7 \pm 60.1	NS
One-year endoscopy	67.6 \pm 75.6	NS
Granule count		
Initial endoscopy	105.5 \pm 58.1	
3-month endoscopy	72.0 \pm 28.2	NS
One- year endoscopy	48.7 \pm 43.3	p<0.05
Mast cells/hpf		
Initial endoscopy	6.4 \pm 12.9	
3-month endoscopy	3.4 \pm 2.7	NS
One- year endoscopy	4.7 \pm 5.7	NS
Langerhans cells/hpf		
Initial endoscopy	3.7 \pm 2.5	
3-month endoscopy	6.8 \pm 3.5	p<0.01
One- year endoscopy	5.5 \pm 2.1	NS

Table 1: Comparison of histological markers in Eosinophilic Esophagitis. *p values are comparing 3-month and 1-year endoscopy to initial endoscopy.

Compared to initial endoscopy, the mast cell number was not different at the 3-month or 1-year follow-up endoscopy. The Langerhans cell number was increased at the 3-month follow-up endoscopy, but was not significantly different from the initial endoscopy at the 1-year follow-up (Table 1).

Comparison between proximal and distal esophageal biopsies

There was no statistically significant difference in eosinophil number between proximal and distal biopsies for all endoscopies. There was no difference in granule number between proximal and distal biopsies on the initial and 3-month follow-up endoscopies. However, at the 1-year follow-up endoscopy, the granule number in the proximal biopsy (50.8 ± 41.8) was significantly increased compared with the distal biopsy (25.1 ± 28.7 , p<0.05, Table 2).

	Proximal esophagus (mean \pm SD)	Distal esophagus (mean \pm SD)	p value
Initial endoscopy	139.2 \pm 84.2	151.0 \pm 10.5	p=NS
3-month endoscopy	61.4 \pm 30.2	46.0 \pm 41.7	p=NS
One year endoscopy	50.8 \pm 41.8	25.1 \pm 28.7	p<0.05

Table 2: Granule counts in proximal and distal esophagus.

Discussion

Our case series suggests that children diagnosed with EoE treated with PPI monotherapy for at least one year have decreased eosinophil degranulation despite persistent eosinophilic inflammation. The persistence of eosinophils in these patients is not surprising since the criteria used for diagnosing EoE is lack of histologic response to PPI therapy. However, the finding of decreased degranulation despite the persistence of eosinophilic inflammation with only PPI therapy has not been previously described.

The decline in granules and improvement in symptoms may be due to an effect of PPI on eosinophil function. Zhang et al. [4] demonstrated that omeprazole significantly inhibited IL-4 stimulated eotaxin-3 protein secretion and m-RNA expression in EoE esophageal cells. Eotaxin-3 is known to be a potent eosinophil chemoattractant, but it also plays a role in activation and degranulation of eosinophils [4]. Although the studies suggest that PPI has an in-vitro effect [4] as well as a clinical benefit [5] in EoE, our current study is the first to associate the clinical improvement with the decrease in eosinophil degranulation. Reduced eosinophil degranulation after PPI is a morphologic finding that correlates with reduced eotaxin secretion. One of the novelties of the current paper lies in the fact that it provides a morphologic correlate of the biochemical findings, and allows a visual window into the mechanism involved. It is likely that eosinophil degranulation contributes to clinical symptoms, perhaps by inducing inflammation and fibrosis [3]. Although our patients who were clinically well still had some degree of free-lying esophageal granules, the significant decrease in granule number suggests that the lesser degree of degranulation is the factor leading to the clinical improvement. The lack of effect of PPI therapy on eosinophil number in our study may reflect the fact that eotaxin-3 may have a quicker or earlier effect on eosinophil degranulation than recruitment, and that longer PPI treatment may eventually lead to decreased tissue eosinophilia.

We noted that the granule number in distal biopsies at 1-year follow-up was significantly more decreased than the granule count in

proximal biopsies. It is known that patients with EoE have dysmotility [2] which may predispose them to reflux-induced disease. One may postulate that the acid-suppressive effect of PPI contributes to improvement in eosinophil degranulation noted in the distal biopsies.

The inflammatory cascade may play a role in EoE [2]. In this study, we found that the mast cell number did not differ between initial and follow-up endoscopies in the EoE group. Langerhans cells were increased at the 3-month biopsy compared with the initial biopsies; however, the numbers were not different between initial and 1-year biopsies.

Eosinophils contain many different toxic granules and it is unclear which specific granule is responsible for the inflammation, remodeling and fibrosis seen in EoE. Free-lying granules serve as a surrogate marker for all released eosinophil products. Therefore, in this study we did not attempt to identify the specific protein seen within the released granules.

This paper examines the extracellular localization of the eosinophil granules in the epithelium, not the protein content of the granules. The tinctural properties of these granules are stable after fixation. Although formalin fixation may theoretically have an effect on eosinophil degranulation at the moment the tissue is placed into formalin, all tissues were treated the same way. Once embedded in paraffin the tissues are stable in a state of "suspended animation" reflecting their morphology at the time of fixation. Degranulation cannot occur once the tissue is embedded in paraffin.

The patients studied were all maintained on a regular unrestricted diet, and therefore, any differences could not be due to changes in

dietary exposure. The patients in our EoE group are distinct from those patients who are thought to have a newly described entity called PPI-responsive esophageal eosinophilia. In that group, patients have increased esophageal eosinophilia but respond histologically to PPI monotherapy [1].

Prospective studies are needed to confirm our findings, to evaluate the precise mechanism in which PPIs decrease eosinophilic degranulation, and the role granules play to explain symptomatic improvement.

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