

Effect of topical beclomethasone on inflammatory markers in adults with eosinophilic esophagitis: A pilot study

Neeti Bhardwaj, M.D., M.S.,¹ Faoud Ishmael, M.D., Ph.D.,² Erik Lehman, M.S.,³ Deborah Bethards, M.D.,⁴ Francesca Ruggiero, M.D.,⁵ and Gisoo Ghaffari, M.D.⁶

ABSTRACT

Background: Topical corticosteroids have proven efficacy in the treatment of eosinophilic esophagitis (EoE) and are considered the cornerstone of therapy.

Objective: To evaluate the effect of topical beclomethasone dipropionate (BDP) therapy on clinical outcomes, esophageal eosinophilia, and other markers of inflammation in patients with EoE.

Methods: Nine subjects with a biopsy-proven diagnosis of EoE were enrolled. In a cross-over design, the subjects were randomly assigned to a sequence of BDP and placebo. Treatment periods were 8 weeks, with a 4-week washout period. The subjects had endoscopic biopsies and blood tests at baseline and after each treatment period. They were instructed to maintain a diary of symptoms. Immuno-histochemical studies were performed for interleukins IL-4, IL-5, IL-13, granulocyte-macrophage colony-stimulating factor (GM-CSF), and transforming growth factor (TGF) beta. Reverse transcription polymerase chain reaction was performed for IL-3, IL-4, IL-5, IL-10, IL-13, IL-17F, IL-25, IL-33, chemokine ligands (CCL)2, CCL5, CCL11, GM-CSF, and TGF-beta levels. The mast cell tryptase (MCT) level was measured in esophageal tissues.

Results: BDP led to a significantly larger decrease in esophageal eosinophilia compared with placebo, but there was no significant change in peripheral eosinophilia and high-sensitivity C-reactive protein between the two groups. The study was not powered enough for us to report a significant improvement in clinical symptoms. There was a significant decrease in tissue IL-13 and MCT levels from baseline to the end of treatment between the treatment and placebo groups. Mean fold decreases in cytokine expression between the baseline and treatment groups were observed for IL-17F, IL-25, CCL2, and CCL5.

Conclusion: Treatment with topical BDP was associated with significant decrease in esophageal eosinophilia, MCT and IL-13. BDP is a potential alternative to fluticasone propionate and budesonide for treatment of EoE. Larger studies are needed to validate these findings.

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Eosinophilic esophagitis (EoE) is a clinicopathologic disorder characterized by marked esophageal eosinophilic infiltration (≥ 15 eosinophils/high power field [hpf]) and esophageal dysfunction, with variable

responses to acid suppression therapy.^{1–3} Diagnostic guidelines published in 2007, and revised in 2011 and 2013, require the presence of clinical symptoms and demonstration of esophageal eosinophilia in the absence of gastroesophageal reflux disease as determined by a therapeutic trial with proton pump inhibitors (PPI) or the use of pH-monitoring studies.^{1,3,4} Half of the patients with symptomatic esophageal eosinophilia show clinicohistologic resolution on PPI therapy.⁵ This clinical entity is called PPI-responsive esophageal eosinophilia. At baseline, patients with PPI-REE and patients with EoE are clinically, endoscopically, and histologically indistinguishable.⁶

EoE seems to be an antigen-driven T-helper (Th) 2 lymphocyte mediated immune process with a multitude of cytokines and chemokines acting as inflammatory mediators.^{7–9} The Th2 cytokines (interleukin [IL]-4, IL-5, and IL-13) are elevated in patients with EoE, and these, in conjunction with eotaxin-3 (chemokine ligands [CCL]26), have been shown to drive the immune response central to the development of EoE.^{10–12} Mast cells also produce several bioactive compounds that can activate eosinophils, and lead to fibrosis.^{13,14} C-reactive protein has been studied in serum and exhaled

From the ¹Division of Pediatric Allergy and Immunology, Department of Pediatrics; ²Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine; ³Department of Public Health, Sciences; ⁴Division of Gastroenterology and Hepatology, Department of Medicine; ⁵Department of Pathology; and ⁶Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Penn State Hershey Medical Center and Penn State University College of Medicine, Hershey, Pennsylvania

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Address correspondence to Neeti Bhardwaj, M.D., Division of Pediatric Allergy and Immunology, Department of Pediatrics, Penn State Hershey Medical Center and Penn State College of Medicine, 500 University Drive, Mail Code HS83, Hershey, PA 17033

E-mail address: nbhardwaj@hmc.psu.edu

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breath concentrate in asthma, but its role in EoE has not been investigated.¹⁵⁻¹⁷

Treatment options for EoE include dietary restrictions and topical and systemic corticosteroids.¹⁸⁻²⁰ Topical corticosteroids have proven efficacy in the treatment of EoE and are considered a first-line therapy. Medications that are otherwise available as inhalers or aqueous nebulizer solutions for asthma are swallowed to coat the esophageal lining and deliver the medication topically.³ The efficacy of topical fluticasone propionate and oral viscous budesonide in treatment of EoE has been established in randomized, placebo controlled trials.²¹⁻²⁶ To our knowledge, there are no controlled studies of the role of beclomethasone dipropionate (BDP) in the treatment of EoE. The aims of this pilot study were the following: (a) to investigate the effect of an 8-week course of topical BDP on clinical symptoms and the eosinophil count on biopsy specimens of patients with EoE; (b) to determine the effect of BDP on the serum levels of eosinophils and high-sensitivity C-reactive protein; and (c) to determine the effect of BDP on a panel of inflammatory markers, including various interleukins, granulocyte-macrophage colony-stimulating factor (GM-CSF), and transforming growth factor (TGF)- β , and mast cell tryptase (MCT) levels in esophageal tissue. We hypothesized that swallowed BDP would lead not only to improvement in symptoms and resolution of eosinophilic inflammation but also to a decrease in markers of tissue inflammation.

METHODS

This study was sponsored by the Foundation of Young Faculty Award of the American College of Allergy, Asthma and Immunology. The study drug, BDP, and a matching placebo were provided by the Department of Public Health, Penn State University. The study protocol was approved by institutional review board of Penn State College of Medicine, Hershey, Pennsylvania. The study protocol was approved by the Penn State's Human Subjects Protection Office (ID 32508). The study is registered in an online public database.²⁷ G. Ghaffari, the principal investigator, contributed to the design and methodology, organized the research team, prepared the initial draft, and approved the final manuscript; N. Bhardwaj is the first author and the corresponding author, contributed to preparation of the draft, updated the references, and approved the final draft; F. Ishmael contributed to the methodology, evaluated and interpreted the immunologic studies, and approved the final draft; E. Lehman contributed to the methodology and statistical analysis, and approved the final draft; D. Bethards contributed to the methodology, performed all the endoscopies, and approved the final draft; F. Ruggiero contributed

to the methodology, evaluated and interpreted the pathologic findings, and approved the final draft.

Inclusion criteria were the following (a) male or female subjects, 18-65 years of age, with a biopsy-proven diagnosis of EoE, and (b) subjects able and willing to provide consent for repeated endoscopies with esophageal biopsies and blood work as per study protocol. We do need to acknowledge here that two subjects included in the study had <15 eosinophils/hpf at the time of enrollment. Both of them had a biopsy-proven diagnosis of EoE before starting topical fluticasone therapy. Although the esophageal eosinophilia improved (and continued to be low during the washout period), these patients were still clinically symptomatic, hence, they were included in the study. Exclusion criteria were the following: (a) subjects with suspected or proven inflammatory bowel disease, malignancy, or collagen-vascular disease; (b) subjects who had used oral, inhaled, or swallowed corticosteroids in the past 3 months; (c) subjects who were pregnant or breast-feeding; (d) subjects who were not able to swallow BDP or who were intolerant to the medication; and (e) subjects with a history of ischemic heart disease, diabetes, or dyslipidemia, unless they had been stable in the past 6 months.

Study Design

The study was designed as a randomized, double-blind, placebo controlled cross-over study. Thirteen subjects who met the criteria as outlined above were enrolled to participate in the study. Four withdrew consent. Nine patients eventually completed the protocol. The subjects had baseline endoscopies with biopsies as well as baseline blood tests. They were randomized into two groups to receive either swallowed BDP (inhalation aerosol 80- μ g, 2 puffs inhalation twice a day) or placebo for 8 weeks based on randomization list provided by a statistician. The subjects were instructed to avoid eating and drinking and to stay in an upright position for 30 minutes after the dose. After a 4-week washout period, the subjects were crossed over to the opposite group for 8 weeks each.

During the screening period of 12 weeks before the treatment periods, the enrolled patients were asked to discontinue all previous topical corticosteroids for EoE and to discontinue dietary restrictions, if any. A physician not involved with the study other than setting up the randomization scheme and medication packets held the randomization key, and did not play any other role in the study to prevent unblinding. The subjects maintained a daily diary that documented drug compliance; symptoms such as dysphagia, heartburn, nausea, vomiting, food impaction; and other medications taken. The subjects were evaluated at 4 weeks

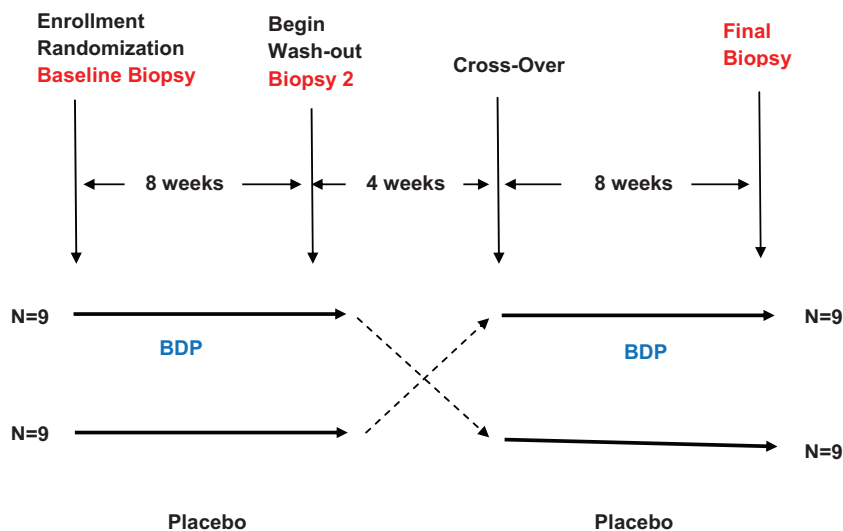


Figure 1. Study design.

during treatment periods for review of symptoms and at the end of treatment (EOT) periods by using peripheral blood testing and endoscopic esophageal biopsy (Fig. 1).

Outcome Measures

The primary outcome was the number of eosinophils in esophageal tissue measured at baseline and at the end of each treatment period. Secondary outcome measures included peripheral blood eosinophil counts, the tissue MCT level, tissue IL-13, CCL2, CCL-5, IL-17F, IL-10, IL-25, and thymic stromal lymphopoietin (TSLP) expression.

Statistical Analysis

The mean difference in pre- and postintervention outcome measures was compared within and between treatment groups by using a linear mixed effects model specifically tailored to analyze data from a 2 × 2 cross-over design. Age and sex effects were added to the models as covariates to adjust for the effect of those factors on the outcome by treatment comparisons, but they were not significant to the models nor did they change the results in a significant way. A paired *t*-test was used to assess the significance of the mean fold change from baseline to the end of the treatment period for tissue expression of other inflammatory markers that could not be measured adequately at the end of the placebo period.

Esophageal Biopsies

The patients were evaluated at Penn State Hershey Medical Center University Physician Endoscopy Center, where an upper endoscopy was performed by one designated physician (D.B.) by using standard proto-

col. A minimum of two biopsy specimens each were obtained from the proximal, middle, and distal esophagus, and were sent for pathologic evaluation per standard protocols. The biopsy specimens were processed routinely and evaluated by using light microscopy (×400) by a pathologist (F.R.).

Immune-Histochemical Staining for MCT and Various Cytokines

The tissue was fixed in 10% neutral buffered formalin and embedded in paraffin, after which 4–5 μ thick sections were cut and mounted onto Plus slides (Fisherbrand, Pittsburgh, PA). These sections were baked for 60 minutes at 60°C, deparaffinized, rehydrated, and submitted to antigen retrieval by using 1 mM EDTA, pH 8.0 for 20 minutes in a steamer, followed by 20 minutes of cooling to room temperature. All staining steps were performed on the Dako Autostainer Plus Agilent Technologies, US Headquarters Santa Clara, CA. Endogenous peroxidase activity was quenched by using 3% hydrogen peroxide for 10 minutes, and the slides were washed with Dako's Wash Buffer. Primary antibodies for MCT (AA1, M7052; Dako North America, Carpinteria, CA) and IL-4, IL-5, IL-13, GM-CSF, and TGF-β (C-19, sc-1292; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), in 1:200 dilution were applied for 60 minutes. By using Dako's Envision + Polymer secondary antibody (30 minutes at room temperature), the Dako DAB+ chromogen (Agilent Technologies) was applied to visualize the staining pattern. The slides were counterstained with Dako's Mayer's hematoxylin (Agilent Technologies), and the coverslip was mounted with Permount (Fisherbrand, Pittsburgh, PA). The staining was interpreted as none, 1+ (weak), 2+ (moderate)

or 3+ (strong), and the numbers of cells were interpreted as low, moderate, and high.

Measurement of Tissue Cytokine Expression: Isolation of RNA from Formalin-Fixed Paraffin- Embedded Tissue and Real-Time PCR

A 3-mm punch biopsy was used to remove a core of tissue from a formalin-fixed paraffin-embedded block, which was then dissolved in xylene. RNA was recovered by using the RecoverAll Total Nucleic Acid Isolation Kit (Life Technologies, ThermoFisher Scientific, Waltham, MA) per the manufacturer's protocol. Total RNA (1 μ g) was reverse transcribed by using the High Capacity cDNA Reverse Transcription Kit (Life Technologies). The complementary DNA was subsequently diluted 1:10, and 2.5 μ L of this was added to 5 μ L of iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) and 2.5 μ L of a specific primer mix (1 μ M). Amplification was quantified by using a MyIQ2 quantitative real time thermocycler (Bio-Rad), and glyceraldehyde-3-phosphate dehydrogenase was used as a reference gene to normalize data. A list of primers used for quantification of gene expression of various cytokines may be found in the supplementary section of the article Paganiban et al.²⁸

RESULTS

Between April 2010 and June 2011, 17 patients were recruited, 13 were randomized. Nine subjects completed the study and were included in the final analysis. The enrolled subjects were randomly assigned to a sequence of BDP and placebo treatments for 8 weeks each with a 4-week washout period in between. There were six men and three women, age range of 18–60 years and mean of 31 years. Seven patients had allergic rhinitis and one also had asthma. Eight patients had food and aeroallergen sensitivities, with positive skin and/or ImmunoCap immunoglobulin E (Quest Diagnostics, Madison, NJ) testing results. Six patients had been on dietary restrictions in the past, but no dietary changes were made during the study period. All the patients had received a trial of PPIs (Table 1) and continued to be on a PPI during the study. Four patients had been on swallowed fluticasone propionate before enrollment in the trial, and, for them, a 12-week washout period before randomization was completed. Compliance as reported on daily diaries was 100%.

Symptoms

All the patients had dysphagia with or without heartburn at the baseline. Other commonly reported symptoms included burping and belching, abdominal pain, nausea, and throat scratching (Tables 2 and 3). The frequency of dysphagia at baseline varied among the patients. Four subjects reported complete resolu-

tion of dysphagia and heartburn while on the study drug. Of these, two had been randomized to BDP first and two to placebo first. Among the remainder of the patients, the frequency of dysphagia and heartburn decreased on the drug but did not resolve completely. Patients with abdominal pain as one of the reported symptoms did not experience any change throughout the study period. One patient may have continued to experience the effects of BDP after being crossed over to the placebo group.

Tissue and Peripheral Eosinophil Count

The data on esophageal and peripheral blood eosinophil counts is presented in Table 1. Tissue eosinophil counts represent the mean of the maximum number of eosinophils detected per biopsy sample. There was a significant decrease in tissue eosinophil count (Fig. 2) from baseline to the EOT within the BDP group (change, -50.68 cells/hpf; $p = 0.006$) but not within placebo group (change, -25.28 cells/hpf; $p = 0.105$). Changes in peripheral blood eosinophil count (Fig. 2) from baseline to EOT in the drug as well as the placebo groups were not significant (change, -0.25 [$p = 0.05$] for the BDP group; and change, -0.34 [$p = 0.412$] for the placebo group). The individual pre- and posttreatment esophageal eosinophil counts for each of the randomized individuals are shown in Fig. 2. Subjects 2, 3, 6, and 7 received drug first, and then placebo after a 4-week washout. The sequence was reversed for subjects 1, 4, 5, 8, and 9.

Tissue MCT and IL-13 Expression

There was a significant decrease in tissue MCT staining (Fig. 3 A) from baseline to EOT within the treatment group (change, -6.79 ; $p < 0.001$) but not within the placebo group (change, -0.16 ; $p = 0.893$). The decrease in tissue IL-13 expression (Fig. 3 B) from baseline to EOT within the BDP group was significant (change, -0.89 ; $p = 0.005$) but not within the placebo group (change, 0.09 ; $p = 0.7$). The reactivity with monoclonal antibodies against IL-4, IL-5, GM-CSF, and TGF- β was generally not observed, and the pathologist (F.R.) considered the response as inconclusive.

Tissue Expression of Other Inflammatory Markers

RNA expression of CCL2, CCL5, IL-25, IL-17F, IL-10, and TSLP was evaluated in esophageal tissue from seven of the enrolled subjects. Decreases in cytokine expression between baseline and treatment groups were observed for IL-17F (3.5-fold; $p = 0.0004$), IL-25 (2.2-fold; $p = 0.03$), CCL2 (1.82-fold; $p = 0.04$), and CCL5 (3.1-fold; $p = 0.004$). The changes in IL-10 and TSLP expression were nonspecific (Fig. 4).

Table 1 Patient data

Patient No.	Age, y	Sex	Atopy*	+SPT/ ImmunoCAP#	Previous Dietary Restrictions	PPI	Previous Long-Term Therapy	Tissue Eosinophil Count (cells/hpf)	Blood Eosinophil Count (cells/hpf)	Baseline		Drug or Placebo First	
										BDP	Placebo		BDP
1	30	M	AR	Yes	Yes	Yes	No	60	0	51	0.1	0.5	Placebo
2	40	M	AR	Yes	Yes	Yes	FP	16	0	40	0.2	0.5	Drug
3	61	M	No	No	No	Yes	FP	14	0	28	0.3	0.1	Drug
4	34	M	AR	Yes	No	Yes	FP	12	22	20	0.2	0.1	Placebo
5	21	F	AR	Yes	No	Yes	No	20	0	0	0.1	0.18	Placebo
6	33	M	AR	Yes	Yes	Yes	FP	85	0	100	1.2	0.2	Drug
7	55	M	AR	Yes	Yes	Yes	FP	120	8	0	0.42	0.25	Drug
8§	19	F	Asthma	Yes	Yes	No	No	100	35	40	0.57	0.3	Placebo
9	26	F	AR	Yes	Yes	Yes	FP	15	0	0	0.11	0.14	Placebo

SPT = Skin-prick test; PPI = proton-pump inhibitor; BDP = beclomethasone dipropionate; AR = allergic rhinitis; FP = fluticasone propionate.

*Patients with atopy had asthma and/or AR.

#Patients were considered food or aeroallergen sensitized if the SPT or the ImmunoCAP Ig E testing result was positive.

§This patient was on montelukast for asthma.

Table 2 Frequency of dysphagia and/or heartburn

Patient No.	Dysphagia and/or Heartburn			
	Baseline	Drug	Placebo	Washout
1*	Daily	0	Daily	Daily
2#	Daily	0	1–2 times/wk	0
3#	0–1 times/wk	0–1 times/wk	1–2 times/wk	0
4*	3 times/wk	0	0–1 times/wk	2 times/wk
5*	Daily	2 times/wk	Daily	0
6#	Daily	2 times/wk	Daily	Daily
7#	2 times/wk	0	1 times/wk	1 times/wk
8*	2 times/wk	2 times/wk	3 times/wk	3 times/wk
9*	Dysphagia, 2 times/wk; heartburn, daily	Dysphagia, 0; heartburn, daily	Dysphagia, 2 times/wk; heartburn, daily	Dysphagia, 0; heartburn, daily

*Received placebo first.

#Received the drug first.

Table 3 Frequency of other symptoms

Patient No.	Symptom	Baseline	Drug	Placebo	Washout
1	Burping and/or belching	Daily	0	0	0
3	Throat scratching and/or hoarseness	0	2 times/wk	1 times/wk	1 times/wk
5	Nausea	Daily	1–2 times/wk	Daily	0
8	Abdominal pain	Daily	Daily	Daily	Daily
9	Abdominal pain, bloating	Daily	Daily	Daily	Daily

DISCUSSION

We reported the results of a randomized, double-blinded, placebo controlled, cross-over trial of the effect of BDP on clinical and histologic findings in adult patients with EoE. To our knowledge, this is the first trial that investigated BDP as a treatment option for EoE. Faubion *et al.*,²⁹ in 1998, reported the case of a 12-year-old boy with EoE who responded to swallowed beclomethasone as part of a case series. So far, fluticasone propionate administered through a metered-dose inhaler and oral viscous budesonide suspension have been shown to induce and maintain low esophageal eosinophil levels safely.^{23,30,31}

All the patients in our trial had dysphagia and heartburn at baseline and all of them reported improvement in the frequency of dysphagia and heartburn symptoms while on BDP but not while on placebo. It needs to be acknowledged, however, that the study was not powered enough for us to report a significant effect of the drug on clinical symptoms. Two patients who had also reported abdominal pain and/or nausea at baseline continued to have these symptoms during the study, although esophageal eosinophilia decreased significantly. Improvement in clinical symptoms does not always correlate with EoE. Results of some studies showed that eosinophilia can improve without a significant change in clinical symptoms.^{26,32,33}

Tissue eosinophilia does not always correlate with clinical presentation but in association with other histologic features; this is the only objective tool for monitoring response to therapy. Our study population underwent a significant decrease in esophageal eosinophil counts on BDP compared with placebo; however, the small study population limited our ability to extrapolate these findings. There was a statistically significant decrease in peak esophageal eosinophil count from baseline to the EOT period in the BDP group ($p = 0.006$). Five patients had complete resolution of esophageal eosinophilia, and, in one patient, the number of eosinophils decreased from 120 to 8 eosinophils/hpf on BDP. Of these, four had been randomized to drug first and then had been crossed-over to placebo. For three of these patients who were “drug-first,” the tissue eosinophil count bounced back up significantly when they were crossed-over to placebo.

Of the nine patients who completed the study, five were randomized to placebo first. Of these, three patients had significant resolution of tissue eosinophilia, which was maintained when crossed over to BDP. These three patients may have had PPI-responsive esophageal eosinophilia, which is clinically, endoscopically, and histologically indistinguishable from EoE.⁶ One subject had been on swallowed fluticasone before and may have continued to experience the lingering

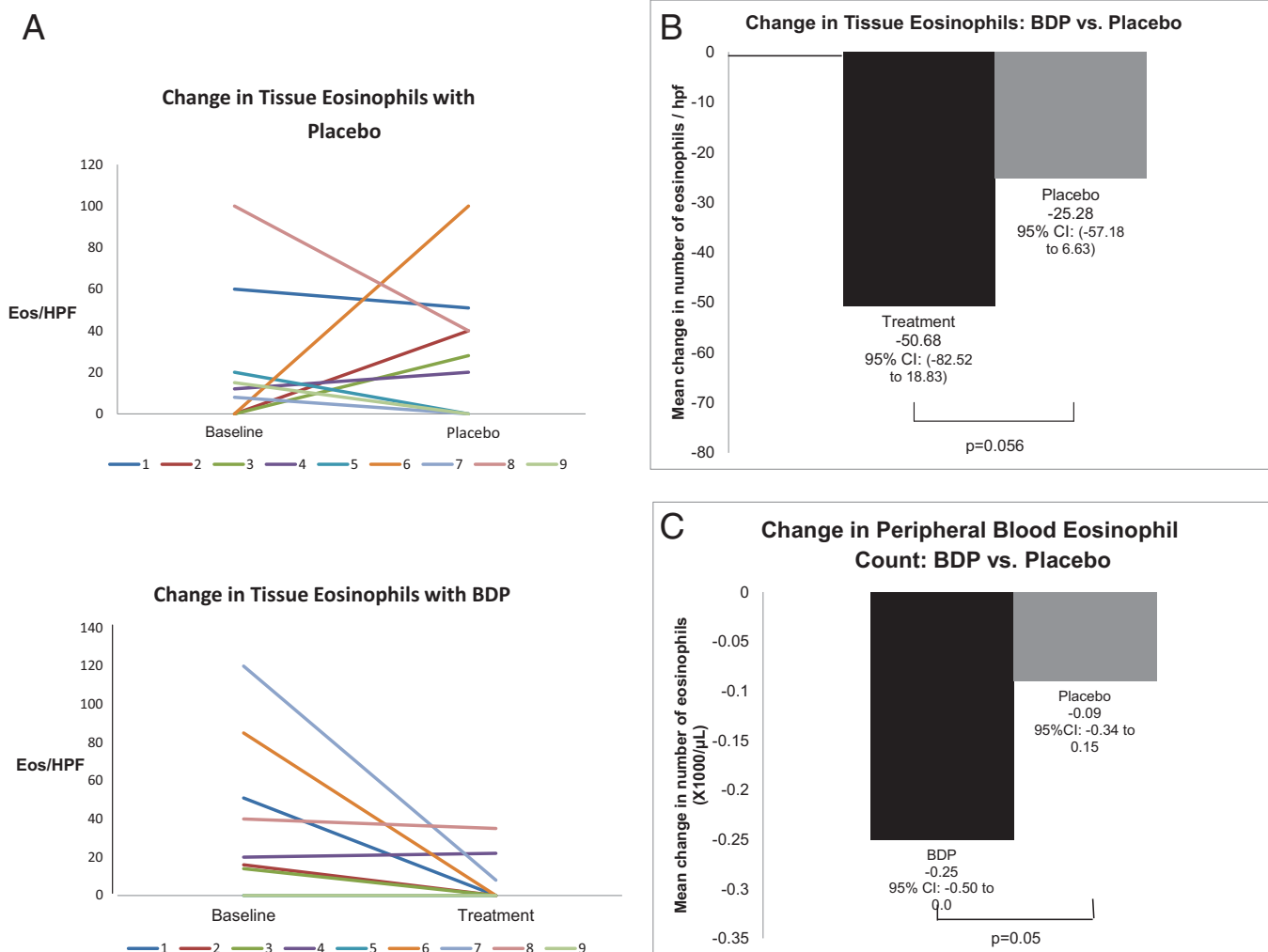


Figure 2. Change in tissue and peripheral blood eosinophilia. (A) Individual pre- and posttreatment peak esophageal eosinophil counts (eosinophils/hpf) after 8 weeks of treatment with beclomethasone dipropionate (BDP) or placebo ($n = 9$ each). Baseline refers to the eosinophil count at the time of entry into the placebo or BDP arms of the trial. Because it was a cross-over trial, some patients who entered the drug arm first had no esophageal eosinophils at baseline at the point of entry into the placebo arm. (B) The mean change in the number of esophageal eosinophils before and after treatment with BDP and placebo for 8 weeks each. (C) The mean change in peripheral blood (absolute) eosinophil counts before and after treatment with BDP or placebo for 8 weeks each.

effects of the fluticasone. For others, a variability in eosinophil counts over time, as seen in asthma, could explain this observation.³⁴ Two subjects continued to have significant tissue eosinophilia as well as clinical symptoms. We need to address the observation that subjects 3 and 4 had <15 eosinophils/hpf at the beginning of the study.

All the subjects enrolled in the study had an established diagnosis of EoE based on biopsy findings of Esophagogastroduodenoscopy undertaken after at least 8 weeks of PPI trial (>15 eosinophils/hpf). The biopsy results noted in Table 1 represent a subsequent Esophagogastroduodenoscopy performed before initiation of the study. These two subjects had previously been treated with swallowed topical fluticasone, which was discontinued in the 3-month washout period be-

fore enrollment in the study. It is possible that the effects of previous topical steroid therapy still lingered. These subjects continued to be symptomatic despite improvement in esophageal eosinophilia and, therefore, were included in the study. Alternatively, it is possible that there could be baseline variability in the number of eosinophils infiltrating the esophagus over time, similar to observations in asthma in which subjects may have intermittent airway eosinophilia.³⁴

There was a modest decrease in the peripheral blood eosinophilia with the BDP treatment, but it did not reach statistical significance ($p = 0.05$). Other studies did not show this parameter as a reliable test to monitor the response to treatment. Peripheral eosinophilia (>300 – 350 eosinophils/ mm^3) has been documented in 40–50% of patients with EoE in previous studies.^{35–37}

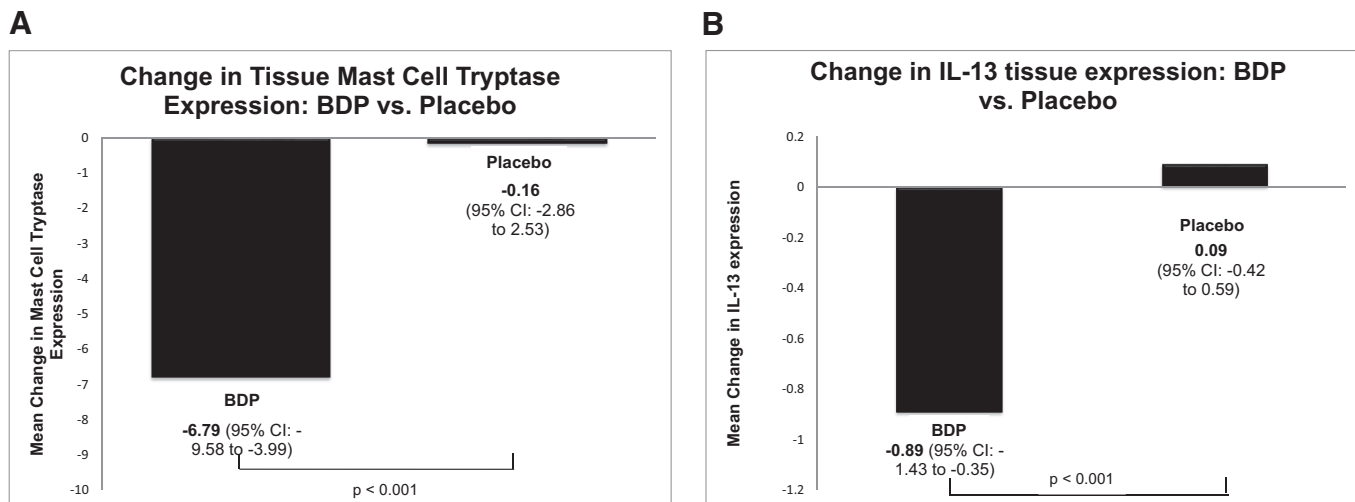


Figure 3. The mean change in esophageal (A) mast cell tryptase level, and (B) interleukin IL-13 expression before and after treatment with beclomethasone dipropionate (BDP) or placebo for 8 weeks each.

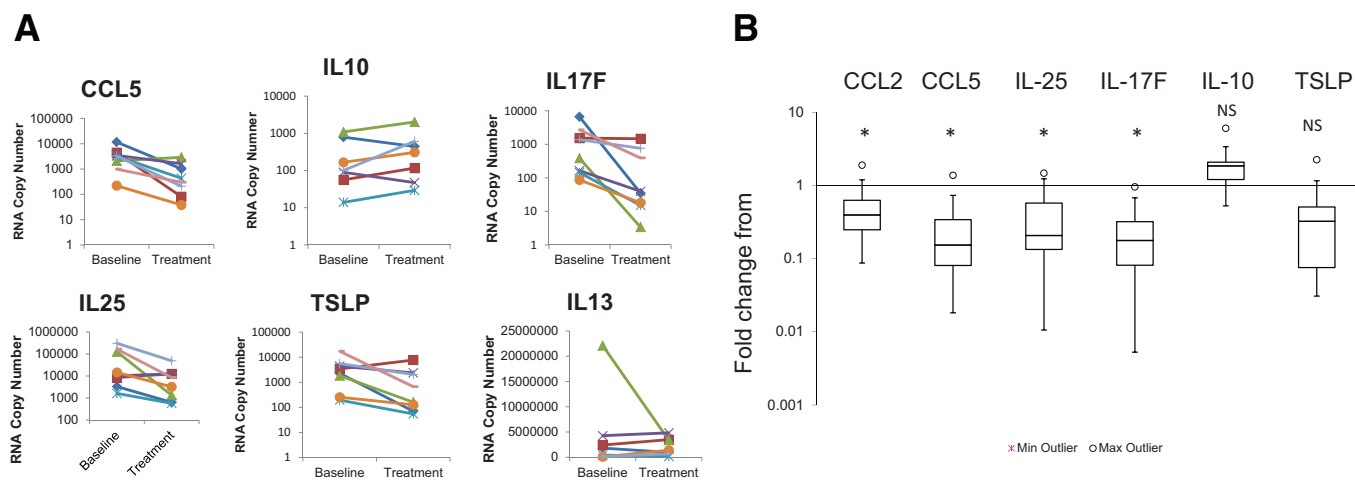


Figure 4. (A) Individual pre- and posttreatment esophageal RNA expression of various inflammatory markers as measured by reverse transcription polymerase chain reaction before and after 8 weeks of topical treatment with beclomethasone dipropionate (BDP). (B) The mean change in RNA expression of various inflammatory markers in the subjects treated with BDP for 8 weeks compared with baseline.

Straumann *et al.*,³⁸ in 2008, showed a statistically significant decrease in peripheral blood eosinophil count with budesonide therapy, however, the shifts in esophageal and peripheral blood eosinophilia were not entirely concordant. We did not find any correlation between peripheral blood high-sensitivity C-reactive protein levels and esophageal eosinophilia.

Numerous studies have established the key role of Th2 pathway cytokines in the development of EoE, with IL-13 being a key cytokine involved in eosinophilic trafficking to inflammatory sites.^{9,11} We found a significant decrease in tissue IL-13 immunohistochemical staining from baseline to EOT within the BDP group ($p = 0.005$) but not within the placebo group ($p = 0.7$). The decrease in IL-13 expression with the BDP treatment may be considered as an objective measurement of response to treatment. Mast cells produce

an abundance of cytokines that activate eosinophils and molecules that directly promote tissue remodeling.¹³ Two unique mast cell-related transcriptomes have been recognized.^{13,14,39} We found a significant decrease in the tissue MCT staining in esophageal tissues from baseline to EOT in the BDP group ($p < 0.001$), whereas the change within the placebo group was not significant ($p = 0.893$).

We further looked at the messenger RNA expression of various other inflammatory markers. For this part of the study, we were able to compare only between the baseline and the treatment groups because there was insufficient tissue from the placebo group. CCL2 and CCL5 are chemokines involved in leukocyte recruitment to inflammatory sites. Their role in EoE has not been investigated. TSLP is a cytokine produced by epithelial cells and targets dendritic cells to secrete

Th2-inducing cytokines and chemokines.⁴⁰ IL-25 also is an epithelium-derived cytokine that promotes airway inflammation and remodeling in asthma.⁴¹ IL-17F is an IL-17 family cytokine that has been shown to be associated with chronic inflammatory lung diseases, including asthma and chronic obstructive pulmonary disease, especially in subjects with atopy.⁴² Little is known about the role of each of these cell products in EoE pathogenesis. In our study, the expression of CCL5 and IL-17F in the treatment group decreased in statistically significant amounts when compared with baseline. There was a modest decrease in CCL2, IL-25, and TSLP expression between treatment and baseline that did not reach statistical significance (Fig. 4). The role of these cytokines as potential end points for therapeutic interventions remains to be elucidated.

CONCLUSION

Topical steroids are the mainstay of EoE treatment in children and adults. Swallowed aerosolized fluticasone and oral viscous budesonide are the commonly used forms.⁴³ Several randomized clinical trials examined the use of topical steroids for EoE.^{20,24–26,44–46} Two systemic reviews with meta-analyses showed an impressive (10- to 13-fold) reduction in mucosal eosinophilia, although the reduction in clinical symptoms ranged from none to threefold only.^{47,48} Most patients relapse if topical steroids are stopped after initial treatment. Our study showed that BDP was an effective treatment option for EoE, based on resolution of clinical symptoms as well as esophageal eosinophilia in patients treated with the drug. No significant adverse effects were reported with the study drug.

Measurement of morning cortisol levels would have been helpful to assess the effect, if any, of swallowed BDP on adrenal function. Dohil *et al.*²⁴ found no difference between pre- and posttreatment morning cortisol levels in children with EoE treated with oral budesonide. A recent study reported adrenal insufficiency in 10% of children treated with swallowed glucocorticoids for ≥ 6 months and was found only in those treated with fluticasone propionate of $>440 \mu\text{g}/\text{day}$.⁴⁹ The changes in tissue mast cell numbers and IL-13 expression also supported our conclusion about its effectiveness as a potential alternative to fluticasone propionate and budesonide for treatment of EoE. Although the 8-week treatment period may not be sufficient, the cross-over design of the study added to the power of the study and made this conclusion reasonable. The major limitation of our study was the small sample size. Larger prospective studies that investigate the effect of the drug for a longer duration of time are warranted.

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