Preliminary Communication

Association of Acute Gastroesophageal Reflux Disease With Esophageal Histologic Changes

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IMPORTANCE The histologic changes associated with acute gastroesophageal reflux disease (GERD) have not been studied prospectively in humans. Recent studies in animals have challenged the traditional notion that reflux esophagitis develops when esophageal surface epithelial cells are exposed to lethal chemical injury from refluxed acid.

OBJECTIVE To evaluate histologic features of esophageal inflammation in acute GERD to study its pathogenesis.

DESIGN, SETTING, AND PARTICIPANTS Patients from the Dallas Veterans Affairs Medical Center who had reflux esophagitis successfully treated with proton pump inhibitors (PPIs) began 24-hour esophageal pH and impedance monitoring and esophagoscopy (including confocal laser endomicroscopy [CLE]) with biopsies from noneroded areas of distal esophagus at baseline (taking PPIs) and at 1 week and 2 weeks after stopping the PPI medication. Enrollment began May 2013 and follow-up ended July 2015.

INTERVENTIONS PPIs stopped for 2 weeks.

MAIN OUTCOMES AND MEASURES Twelve patients (men, 11; mean age, 57.6 year [SD, 13.1]) completed the study. Primary outcome was change in esophageal inflammation 2 weeks after stopping the PPI medication, determined by comparing lymphocyte, eosinophil, and neutrophil infiltrates (each scored on a 0-3 scale) in esophageal biopsies. Also evaluated were changes in epithelial basal cell and papillary hyperplasia, surface erosions, intercellular space width, endoscopic grade of esophagitis, esophageal acid exposure, and mucosal impedance (an index of mucosal integrity).

RESULTS At 1 week and 2 weeks after discontinuation of PPIs, biopsies showed significant increases in intraepithelial lymphocytes, which were predominantly T cells (median [range]: 0 (0-2) at baseline vs 1 (1-2) at both 1 week [P = .005] and 2 weeks [P = .002]); neutrophils and eosinophils were few or absent. Biopsies also showed widening of intercellular spaces (confirmed by CLE), and basal cell and papillary hyperplasia developed without surface erosions. Two weeks after stopping the PPI medication, esophageal acid exposure increased (median: 1.2% at baseline to 17.8% at 2 weeks; Δ, 16.2% [95% CI, 4.4%-26.5%], P = .005), mucosal impedance decreased (mean: 2671.3 Ω at baseline to 1508.4 Ω at 2 weeks; Δ, 1162.9 Ω [95% CI, 629.9-1695.9], P = .001), and all patients had evidence of esophagitis.

CONCLUSIONS AND RELEVANCE In this preliminary study of 12 patients with severe reflux esophagitis successfully treated with PPI therapy, stopping PPI medication was associated with T lymphocyte–predominant esophageal inflammation and basal cell and papillary hyperplasia without loss of surface cells. If replicated, these findings suggest that the pathogenesis of reflux esophagitis may be cytokine-mediated rather than the result of chemical injury.

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Approximately 20% of adult Americans have symptoms of gastroesophageal reflux disease (GERD).[1] The conceptual framework for GERD pathogenesis emerged from a 1935 *JAMA* report by Winkelstein that described patients with heartburn and inflammation in the distal esophagus and proposed that they had "peptic esophagitis ... resulting from the irritant action on the mucosa of free hydrochloric acid and pepsin."[2] This concept, that reflux esophagitis develops as an acid-peptic chemical injury, has been largely unchallenged. The esophageal histologic abnormalities thought to be typical of GERD (basal cell hyperplasia, elongation of connective tissue papillae, infiltration by neutrophils and eosinophils) have been attributed to refluxed gastric acid-related chemical injury to esophageal epithelial cells starting at the luminal surface. The acid-induced death of surface cells is assumed to stimulate hyperplasia of basal progenitor cells, make papillae appear elongated, and attract granulocytes.[3-6]

CLE *confocal laser endomicroscopy*

GERD *gastroesophageal reflux disease*

GERD-HRQL *GERD Health-Related Quality of Life*

HPF *high-power field*

PPI *proton pump inhibitor*

An earlier study in rats found that reflux esophagitis did not develop as a chemical injury starting at the epithelial surface, but rather began with a submucosal infiltration by lymphocytes that later progressed upward to the epithelial surface.[7] Basal cell hyperplasia and papillary elongation were observed to precede surface cell damage, and it was noted that brief exposures to acid and bile salts did not kill human esophageal cells in culture, but stimulated them to secrete inflammatory cytokines.[7,8] Thus, an alternative concept for GERD pathogenesis was proposed in which refluxed gastric material did not damage esophageal epithelial cells directly, but stimulated them to secrete cytokines that attracted immune cells, which ultimately damaged the mucosa.[7]

Patients typically have GERD symptoms for years before seeing a physician,[9] and early features of reflux esophagitis have not been evaluated prospectively in humans. Studies have shown that erosive esophagitis successfully treated with proton pump inhibitors (PPIs) usually returns within 6 to 12 months after stopping PPI medication,[10,11] but the rapidity with which esophagitis redevelops is not clear. We hypothesized that acute reflux esophagitis could be induced by briefly interrupting PPI therapy in patients with severe erosive esophagitis successfully treated with PPIs. The aim of this study was to evaluate the histologic features of esophageal inflammatory changes in acute GERD.

Methods

This study was approved by Dallas Veterans Affairs Medical Center's institutional review board. Patients provided written informed consent and were compensated for study participation.

Study Population and Design

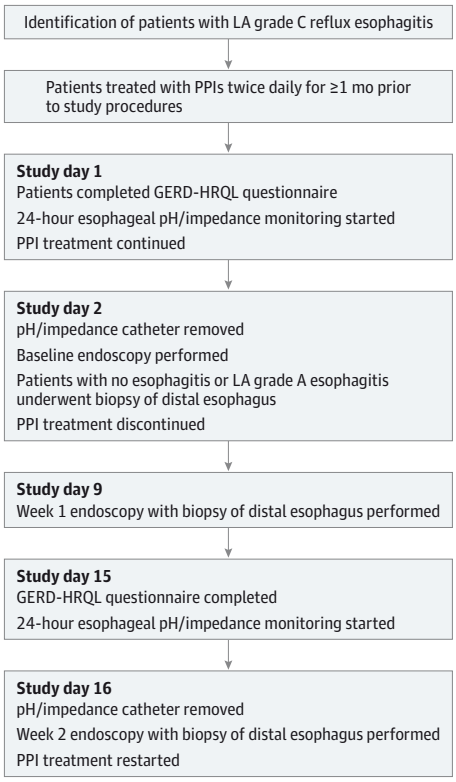
The endoscopy database of the Dallas Veterans Affairs Medical Center was searched for patients with Los Angeles grade C (LA-C) reflux esophagitis diagnosed between December 2011 and January 2014 (LA grades: 0 = no esophagitis; A = ≥1 mucosal break ≤5 mm long not extending between mucosal folds; B = ≥1 mucosal break >5 mm long not extending between mucosal folds; C = ≥1 mucosal break continuous between the tops of ≥2 mucosal folds, involving <75% of the circumference; D = ≥1 mucosal break involving ≥75% of the circumference).[12] Two gastroenterologists (K.B.D., S.J.S.) reviewed endoscopic images, and invited patients with verified LA-C esophagitis to participate. Exclusion criteria included history of esophageal varices, esophageal or gastric surgery, non-GERD esophageal disease, coagulopathy, anticoagulant usage, pregnancy, and comorbidity precluding safe participation. Enrollment began May 2013; follow-up ended July 2015.

Patients with LA-C esophagitis were treated with PPIs twice daily for 1 month or more (**Figure 1**). On study day 1, patients took their morning PPI and completed the GERD-Health-Related Quality of Life (HRQL) questionnaire (a validated instrument for GERD symptom severity; score range, 0 [no symptoms] to 50 [worst symptoms]).[13] Esophageal manometry and 24-hour pH and impedance monitoring were performed with a pH electrode positioned 5 cm above the lower esophageal sphincter. Patients took a PPI that evening and the next morning, when esophagoscopy was performed using both high-definition white light endoscopy (Olympus Medical) and confocal laser endomicroscopy (CLE; Pentax Medical). Patients with LA-B, LA-C, or LA-D esophagitis were not eligible for further study. Patients with no esophagitis or LA-A esophagitis had 4 esophageal biopsies obtained 1 cm to 3 cm proximal to the squamocolumnar junction for histologic evaluation. PPIs were then stopped; patients were given antacids for heartburn. On day 9, esophagoscopy was performed for LA grading and biopsy. On day 15, patients completed another GERD-HRQL questionnaire and had pH and impedance monitoring. The next day, esophagoscopy was performed for LA grading and biopsy, and patients resumed PPI therapy. During the second and third esophagoscopy, care was taken to avoid obtaining biopsies from prior biopsy sites or mucosal breaks.

CLE Procedures

During CLE, which provides 1000-fold magnification of esophageal mucosa, patients were given fluorescein sodium (5 mL) intravenously to enhance identification of cells and capillaries. Images were acquired from distal (1-3 cm above squamocolumnar junction) and proximal esophagus (10 cm above squamocolumnar junction). After the procedure, an investigator (K.B.D.) who was blinded to procedure time point reviewed all images and chose 2 images from the proximal and distal esophagus that were technically best suited for intercellular space and capillary width measurements by ImageJ software (National Institutes of Health), version 1.48, using the mean of 10 measurements of the widest intercellular spaces seen and of any capillaries seen.

Figure 1. Study Design



LA indicates Los Angeles; GERD-HRQL, Gastroesophageal Reflux Disease Health-Related Quality of Life; PPI, proton pump inhibitor.

Resting Esophageal Mucosal Impedance

Resting esophageal mucosal impedance reflects electrical conductivity of the esophageal wall and is an index of mucosal integrity.[14-16] Resting impedance was measured at the start of each impedance and pH monitoring period, at a level 5 cm above lower esophageal sphincter.

Histologic Procedures

Histologic features were assessed by consensus of 2 study pathologists (A.T.A., R.D.O.) blinded to endoscopic order and findings. Formalin-fixed, H&E-stained biopsies were scored on a 0 through 3 scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe) for (1) type and degree of epithelial inflammation (lymphocytes, eosinophils, neutrophils), (2) basal cell and papillary hyperplasia, and (3) spongiosis (dilated intercellular spaces). In the absence of a validated system for scoring inflammation in esophageal biopsies, this scale was used based on a similar, stepwise, 4-point grading system for scoring inflammatory activity in the colon.[17] The study protocol erroneously specified use of a 0 through 4 scale, but the study pathologists used a 0 through 3 scale to score inflammation as was originally planned. Quantitative assessment of inflammatory cell density was performed by counting peak number of lymphocytes, neutrophils, and eosinophils per high-power field (HPF, 40× = 0.238 mm^2) in each of the 3 most representative and best-oriented biopsy fragments. Immunoperoxidase studies were performed on an autostainer (Leica Bond III), using a polymer refine detection kit (catalog No. DS9800) and heat-induced epitope retrieval, using either a citrate-based pH 6.0 (Bond Epitope Retrieval Solution 1; AR9961) or pH 9.0 (Bond Epitope Retrieval Solution 2; AR9640) epitope retrieval solution for pan-T cell marker CD3 (Dako A0452, heat-induced epitope retrieval 2 for 20 minutes, 1:250) and B cell marker CD20 (Dako M0755, heat-induced epitope retrieval 1 for 30 minutes, 1:500). Slides were incubated in primary antibody for 30 minutes, followed by secondary polymer for 15 minutes. 3,3′-Diaminobenzidine was used for visualization.

Outcomes

The study protocol did not clearly specify primary and secondary outcomes, and cited study purpose as "to elucidate the early histological events in the pathogenesis of reflux esophagitis in patients with GERD, and to correlate those events with esophageal expression of HIF-2α and pro-inflammatory cytokines, and with changes in esophageal proliferation" (Supplement 1). ClinicalTrials.gov cites study purpose as "to determine the role of HIF [hypoxia-inducible factors]-2α on the production of inflammatory cytokines that lead to reflux esophagitis," and lists the primary and secondary outcome measures as "[c]hange in esophageal inflammation from baseline to 14 days" and "[c]hange in HIF-2α levels from baseline to 14 days," respectively.

To determine change in esophageal inflammation histologically, lymphocytic, eosinophilic, and neutrophilic infiltration in esophageal biopsies (scored on the 0-3 scale described above) at baseline, 1 week, and 2 weeks were compared. In addition, changes in GERD-HRQL symptom scores, esophageal acid exposure (percentage of total time esophageal pH<4), endoscopic grade of esophagitis, esophageal mucosal impedance, and changes in epithelial basal cell and papillary hyperplasia, spongiosis, surface erosions, and intercellular space width were evaluated. These were listed as "procedures to be performed" in the study protocol, but were not specified as outcome measures.

Statistical Methods

Continuous parameters are reported as mean (SD), ordinal parameters as median and range, discrete parameters as N and percentage. Continuous dependent variables were tested for normality with the Shapiro-Wilk test. Normally distributed continuous parameters were compared with paired samples *t* tests and repeated measures multivariate analysis of variance with least significant difference multiple comparisons. Non-normally distributed continuous parameters and ordinal parameters were compared with Wilcoxon signed-rank and Friedman tests. Binary dependent variables were tested with repeated measures logistic regression (generalized linear models with logit functions). Analyses were performed with SPSS (IBM), version 22.0, for Windows. Study α was .05, with all tests reflecting 2-tailed comparisons.

Sample Size Calculation and Power Analysis

The study protocol specified that a power analysis performed using SAS (SAS Institute), version 9.2, indicated that 12 participants were required to achieve study aims, based on a 1-sample repeated measures analysis of inflammation over time with an anticipated effect size of 80%, a study α of .05, β of .10, 2-tailed. "Effect size of 80%" meant that no histologic evidence of esophageal inflammation (grade 0) was anticipated in any patient at baseline, and some degree of histologic inflammation (grade 1-3) was expected in 80% of patients by 2 weeks after stopping PPI medication. These estimates were based on studies in an animal model of reflux esophagitis and on studies of patients with GERD who redeveloped esophagitis within 6 through 12 months of stopping PPI medication.[7,10,11] An attrition rate of 40% (due to dropouts, inability to remain off PPIs, esophagitis on initial endoscopy, etc) was estimated, and enrollment of up to 30 patients was planned to have 12 patients complete the study.

Results

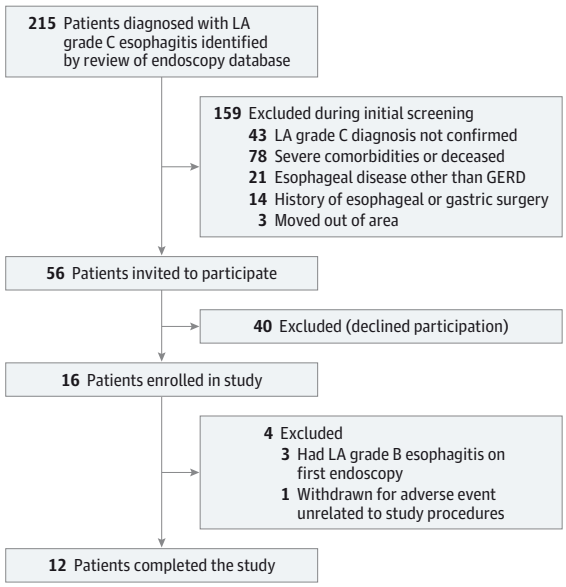
Endoscopy database review identified 215 patients diagnosed with LA-C esophagitis (**Figure 2**); 159 were eliminated by initial screening and 56 were invited to participate; 40 declined. The attrition rate was lower than estimated; it was necessary to enroll only 16 patients to attain 12 who completed the study; 3 patients were excluded because baseline endoscopy showed LA-B esophagitis; 1 was withdrawn due to an adverse event unrelated to study procedures. Enrollment was terminated when the 12th patient completed study procedures. Among the 12 study patients (men, 11; mean age, 57.6 years [SD, 13.1]), 8 took pantoprazole (40 mg twice daily) and 4 took omeprazole (40 mg twice daily) during the month before study procedures.

GERD Symptoms, Esophageal Acid Exposure, Mucosal Impedance, and Endoscopic Findings

GERD-HRQL symptom scores increased from a median of 2 at baseline taking PPIs to 11.5 at 2 weeks after stopping PPIs (median Δ, 4.5 [95% CI, 2.0-12.0]; P = .008) (**Table 1**). Esophageal pH monitoring data were available both at baseline and 2 weeks after stopping PPIs for 10 of the 12 patients. Acid exposure increased from median 1.2% at baseline to 17.8% at 2 weeks after stopping PPIs (median Δ, 16.2% [95% CI, 4.4%-26.5%], P = .005). Mucosal impedance decreased from mean 2671.3 Ω at baseline to 1508.4 Ω at 2 weeks after stopping PPIs (Δ, 1162.9 Ω [95% CI, 629.9-1695.9], P = .001).

At baseline, 11 of 12 patients had no visible esophagitis; 1 had LA-A esophagitis (Table 1). Without PPIs, esophagitis grade increased in 10 patients by week 1, and in 11 patients by week 2; 5 patients developed severe (LA-C) esophagitis (**Figure 3**). By assigning numerical values to LA grades (0 = 0, A = 1, B = 2, C = 3), it was determined that esophagitis increased significantly from baseline (median, 0) to week 1 (median, 1.5; P = .006), and from week 1 to week 2 (median, 2; P = .02).

Figure 2. Flow of Patients Through the Study



LA indicates Los Angeles; GERD, gastroesophageal reflux disease.

Histologic Findings and CLE Measurements

At baseline, there was minimal histologic inflammation (**Table 2**, Figure 3). At 1 and 2 weeks after stopping PPIs, significant increases were noted in ordinal (0-3) histologic scores for intraepithelial lymphocytic infiltration (median [range], 0 [0-2] at baseline to 1 [1-2] at both 1 week [P = .005] and 2 weeks [P = .002]), basal cell and papillary hyperplasia (median [range], 0.5 [0-1] at 1 week to 2 [1-3] at 2 weeks, P < .01), and spongiosis (median [range], 0.5 [0-1] at 1 week to 2 [1-3] at 2 weeks, P < .01). Neutrophils were found only in infrequent areas of microerosion; the median maximum number of neutrophils per HPF in any biopsy specimen at any time was 0 (range, 0-31). Eosinophils also were few in number in most patients at all time points; the median maximum number of intraepithelial eosinophils per HPF in any biopsy specimen at any time was 1.5 (range, 0-9). Lymphocytes were the predominant inflammatory cell type at all time points; the median maximum number of intraepithelial lymphocytes per HPF in any biopsy at any time was 51.5 (range, 26-163). Immunostaining showed that these lymphocytes were almost exclusively CD3+ T cells (eFigure in Supplement 2).

After stopping PPI medication, CLE revealed dilated intercellular spaces containing increased amounts of fluorescein (Figure 3). At weeks 1 and 2, intercellular space width in proximal and distal esophagus, and capillary width in distal esophagus had increased significantly from baseline values (Table 2).

Discussion

In patients with severe erosive reflux esophagitis successfully treated with PPIs, this study showed that interrupting

Table 1. Clinical Features at Baseline (Taking PPIs) and at 1 and 2 Weeks After Stopping PPI Medication

	GERD-HRQL Questionnaire Score[a]		Time Esophageal pH<4, %		Esophageal Impedance, Ω[b]		LA Grade (Numerical Value)[c]		
Patient No.	Baseline	2 Weeks	Baseline	2 Weeks	Baseline	2 Weeks	Baseline	1 Week	2 Weeks
1	0	9	0.3	26.8	NA	650	0 (0)	A (1)	C (3)
2	25	22	10.7	15.1	2249	773	0 (0)	B (2)	B (2)
3	17	29	1.2	23.6	3993	1329	0 (0)	B (2)	B (2)
4	1	3	0.0	21.6	1422	1113	0 (0)	B (2)	B (2)
5	19	20	6.5	6.8	3676	1670	0 (0)	B (2)	C (3)
6	11	16	4.8	18.6	2004	1231	0 (0)	B (2)	C (3)
7	0	14	0.6	16.9	3271	2660	0 (0)	A (1)	C (3)
8	2	4	37.0[d]	88.3[d]	983[d]	1021[d]	0 (0)	0 (0)	A (1)
9	0	5	1.9	37.4	2130	1784	A (1)	0 (0)	A (1)
10	2	4	0.1	16.1	2947	2000	0 (0)	A (1)	B (2)
11	4	18	1.2	NA	2000	850	0 (0)	A (1)	C (3)
12	0	4	0.5	5.6	3021	1674	0 (0)	B (2)	A (1)
Median (range) or mean (SD)	2 (0-25)	11.5 (3-29)	1.2 (0-10.7)	17.8 (5.6-37.4)	2671.3 (832.8)	1508.4 (571.3)	0 (0-1)	1.5 (0-2)	2 (1-3)
Difference baseline vs 2 weeks, median (95% CI)	4.5 (2.0-12.0)		16.2 (4.4-26.5)		1162.9 (629.9-1695.9)[e]				
P value	.008[f]		.005[f]		.001[g]			.006 vs baseline[f]	.02 vs 1 week[f]

Abbreviations: GERD-HRQL, Gastroesophageal Reflux Disease Health-Related Quality of Life; LA, Los Angeles; LES, lower esophageal sphincter; NA, data not available for technical reasons; PPI, proton pump inhibitor.

[a] GERD-HRQL is a validated instrument used to assess the symptomatic response to GERD treatments.[13] The patient is asked to rank 10 GERD symptom questions (eg, How bad is your heartburn? Does heartburn wake you from sleep?) on a scale of 0 (no symptoms) to 5 (incapacitating symptoms); thus, the possible range of scores is 0 to 50. No minimally important difference in GERD-HRQL scores has been defined. In clinical trials, a positive outcome generally is defined as 50% or higher improvement in GERD-HRQL scores.[18]

[b] Esophageal impedance (electrical resistance) was measured at the start of the monitoring period at a level 5 cm above the LES.

[c] LA endoscopic esophagitis grades: 0 = no esophagitis, A = 1 or more mucosal break of 5 mm or less in length not extending between the tops of mucosal folds, B = 1 or more mucosal break more than 5 mm long not extending between the tops of mucosal folds, C = 1 or more mucosal break continuous between the tops of 2 or more mucosal folds, involving less than 75% of the circumference. For statistical comparisons, numerical values were assigned to LA grades (0 = 0, A = 1, B = 2, C = 3).

[d] Due to technical difficulties in identifying the LES in this patient, the pH electrode was inadvertently positioned in the stomach rather than the esophagus. This incorrect positioning also resulted in spurious values for impedance. Consequently, these data were not included in the group analysis of the pH monitoring and impedance results.

[e] Mean (95% CI).

[f] Wilcoxon signed-rank test.

[g] Paired samples *t* test.

PPI therapy was followed by rapid development of acute GERD associated with a significant increase in esophageal acid exposure and significant decrease in mucosal integrity. Within 1 week of stopping PPI medication, most patients redeveloped erosive esophagitis associated with dilation of esophageal intercellular spaces and capillaries. Histologically, this acute GERD was a T lymphocyte–predominant form of inflammation, with minimal involvement by neutrophils and eosinophils. Furthermore, esophageal basal cell and papillary hyperplasia developed in areas without surface erosions. If the traditional notion were true, that acute GERD is caused by refluxed acid directly inflicting lethal, chemical injury to surface epithelial cells, then basal cell and papillary hyperplasia would have been expected only in areas with surface erosions, and the infiltrating inflammatory cells would have been granulocytes primarily.[6]

Apical membranes of esophageal squamous epithelial cells are highly impermeable to hydrogen ions, unlike their basolateral membranes, which are highly acid-permeable.[19] According to the traditional notion of GERD pathogenesis, esophagitis starts when refluxed acid and pepsin initiate damage to proteins of junctional structures binding esophageal epithelial cells to one another.[20,21] These structures normally form a barrier to paracellular diffusion of acid and, when damaged, refluxed acid can diffuse into intercellular spaces to enter epithelial cells through their vulnerable basolateral membranes. Once inside esophageal cells, acid was thought to kill them by denaturing vital proteins, by activating phospholipases and endonucleases, and by interfering with cell respiration.[22,23] This lethal acid injury was assumed to start at the esophageal luminal surface, inducing an acute inflammatory response characterized by epithelial infiltration with granulocytes. The acid-induced death of surface cells was assumed to stimulate hyperplasia of squamous basal progenitor cells and to be associated with elongated and hyperplastic papillae.[3,5] With persistent reflux inducing more epithelial cell death, inflammation was thought to progress into lamina propria and, with ulceration, into submucosa.[6] Thus, acute reflux esophagitis was assumed to develop as an acid-peptic burn progressing from luminal surface through to submucosa. However, this pattern of injury was not observed in the present study.

Figure 3. Representative Images of the Distal Esophagus From a Single Patient at Baseline and at 1 Week and 2 Weeks After Discontinuation of PPI Therapy

LA indicates Los Angeles; PPI, proton pump inhibitor. At baseline, HD-WLE showed an irregular tongue of columnar mucosa (Barrett esophagus) in the 12 o'clock position, but no esophagitis. At day 9, HD-WLE showed long linear mucosal breaks (4 and 6 o'clock positions) extending up the esophagus from the gastroesophageal junction (LA grade B esophagitis). At day 16, HD-WLE showed long mucosal breaks continuous between the tops of mucosal folds (LA grade C esophagitis). CLE revealed fluorescein within bright intraepithelial capillaries (yellow arrowheads), with fluorescein that leaked from blood vessels into intercellular spaces surrounding individual cells, creating a reticular appearance characteristic of squamous epithelium. CLE measurements revealed widened intercellular spaces with increased intercellular fluorescein at days 9 and 16. In the photomicrographs at day 9 and 16, black arrowheads indicate some of the numerous intraepithelial lymphocytes. Note the prominent lymphocytosis, basal cell hyperplasia, and papillary elongation at 2 weeks after discontinuation of PPI therapy. All photomicrographs are H&E stains, original magnification ×20. All images were modified in Photoshop to remove patient identification data, and to enhance clarity. Any adjustments in contrast, color balance, brightness or sharpness were applied to the entire image.

Table 2. Histologic Findings and Confocal Laser Endomicroscopy Measurements

Histologic Finding[a]	Baseline (With PPIs), Median (Range)	Week 1 (Without PPIs), Median (Range)	Absolute Difference for Baseline vs Week 1 (95% CI)	*P* Value	Week 2 Without PPIs, Median (Range)	Absolute Difference for Baseline vs Week 2 (95% CI)	*P* Value
Intraepithelial							
Lymphocytes	0 (0 to 2)	1 (1 to 2)	0.67 (0.25 to 1.08)	.005	1 (1 to 2)	0.58 (0.26 to 0.91)	.002
Neutrophils	0 (0)	0 (0 to 2)	0.17 (−0.20 to 0.53)	.32	0 (0 to 2)	0.25 (−0.14 to 0.64)	.18
Eosinophils	0 (0 to 1)	0 (0 to 1)	0.08 (−0.10 to 0.27)	.32	0 (0 to 1)	0.08 (−0.10 to 0.27)	.32
Basal cell and papillary hyperplasia	0.5 (0 to 1)	2 (1 to 3)	1.25 (0.86 to 1.64)	.002	2 (1 to 3)	1.42 (0.91 to 1.92)	.003
Spongiosis (dilated intercellular spaces)	0.5 (0 to 1)	2 (1 to 3)	1.17 (0.80 to 1.53)	<.001	2 (1 to 3)	1.25 (0.86 to 1.65)	<.001

CLE Measurements[b]	Mean (SD)	No. of Patients	Mean (SD)	No. of Patients		Mean (SD)	No. of Patients		
Intercellular space, μm									
Distal esophagus	3.2 (0.6)	8	4.0 (0.5)	8	0.82 (0.10 to 1.54)	.031	5.1 (1.1)	8	1.91 (1.00 to 2.81)	.002
Proximal esophagus	3.5 (0.5)	8	4.7 (1.3)	7	1.32 (0.05 to 2.60)	.04	5.7 (1.2)	7	2.53 (1.62 to 3.45)	.001
Capillary width, μm									
Distal esophagus	11.0 (1.6)	7	14.1 (3.0)	7	3.03 (0.76 to 5.30)	.02	15.0 (2.6)	6	3.87 (0.86 to 6.88)	.02
Proximal esophagus	10.9 (3.5)	6	13.8 (1.6)	6	3.30 (−0.55 to 7.14)	.08	13.1 (2.5)	6	3.03 (0.78 to 5.28)	.02

Abbreviations: CLE, confocal laser endomicroscopy; PPI, proton pump inhibitor.

[a] Histologic findings are scored on a scale of 0 through 3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe). Comparisons were made using Wilcoxon signed-rank tests.

[b] Technical issues (excessive motion artifact, equipment malfunction, patient inability to tolerate the large CLE endoscope) precluded obtaining CLE measurements in all patients at all time points. CLE measurements were made using ImageJ software (National Institutes of Health), version 1.48. Comparisons were made using paired samples *t* tests.

The results of this study in GERD patients are consistent with those described in another report on a rat model of reflux esophagitis in which it was proposed that refluxed gastric juice might not damage the esophagus directly, but rather incited a cytokine-mediated inflammatory response that ultimately caused esophageal damage.[7] Elevated esophageal levels of pro-inflammatory cytokines have been found in GERD patients, although it remains unclear whether those cytokines are a cause or effect of esophageal inflammation.[24-28] In earlier studies, it was found that acidic bile salts stimulated human esophageal squamous epithelial cells in culture to secrete potent pro-inflammatory cytokines (interleukin [IL]-8 and IL-1β) and that conditioned media from those cells increased migration of inflammatory cells in a migration assay system.[7,8] Cytokines like IL-8 and IL-1β also have proliferative effects,[29,30] which might have contributed to esophageal basal cell and papillary hyperplasia observed in the absence of surface erosions. In esophageal epithelial cells in culture, moreover, PPIs inhibit secretion of IL-8 through acid-independent mechanisms.[8,31] This observation raises the interesting possibility that anti-inflammatory PPI effects, independent of their effects on acid inhibition, might contribute to GERD healing by PPIs.

In the present study, biopsies were taken purposely from areas of distal esophagus that had no visible erosions. Biopsies of eroded areas undoubtedly would have revealed prominent neutrophilic infiltrates, which develop in erosions of virtually any etiology.[32] The finding of neutrophils in such biopsies would provide no useful information about the pathogenesis of the erosions. Biopsies of noneroded esophageal epithelium after PPI interruption revealed infiltration by T lymphocytes with basal cell hyperplasia and papillary elongation, consistent with the proposal that acute GERD is primarily a cytokine-mediated process.[7] Further studies are needed to establish that cytokines are indeed the cause of the histologic changes observed, but our findings suggest that those changes do not appear to be caused by acid-induced death of surface cells.

Dilation of esophageal intercellular spaces is a characteristic GERD feature, and intercellular space dilation was observed (by CLE and histology) as reflux esophagitis progressed in study patients. It has been proposed that this dilation results from an acid-induced increase in epithelial permeability that enables chloride anion and water in the esophageal lumen to enter and expand the intercellular space.[33] The CLE observation that GERD is associated with an increase in blood-borne fluorescein in intercellular spaces raises the possibility that reflux-induced esophageal inflammation might increase esophageal vascular permeability. If so, then leakage of fluid from inflammation-damaged esophageal blood vessels also might contribute to dilation of intercellular spaces in GERD.

There are limitations to this study. Most of the eligible patients declined to participate in this rigorous protocol. The study included only patients with severe (LA-C) reflux esophagitis whose esophagitis had been successfully treated with PPIs; 11 of the 12 patients were men, and all had hiatal hernias. Among individuals with typical GERD symptoms, less than 50% have endoscopic evidence of reflux esophagitis, and less than 20% of those have esophagitis of LA-C grade severity.[34,35] Thus, it is not clear that findings in the study patients are applicable to the general population of patients

with GERD of lesser severity. Study patients had recurrent, acute GERD induced by stopping PPI medication, and it is not clear that findings in those patients are applicable to new-onset GERD occurring spontaneously in untreated individuals. In addition, there was subjectivity involved in choosing CLE images for analysis, which might have introduced bias in interpretation of the CLE data. Finally, it is possible that the drop in mucosal impedance after stopping PPI medication was caused by liquid in the esophageal lumen rather than by impairment of mucosal integrity.

Conclusions

In this preliminary study of 12 patients with severe reflux esophagitis successfully treated with PPI therapy, stopping PPI medication was associated with T lymphocyte–predominant esophageal inflammation and basal cell and papillary hyperplasia without loss of surface cells. If replicated, these findings suggest that the pathogenesis of reflux esophagitis may be cytokine-mediated rather than the result of chemical injury.

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