The prospective study on the effect of ilaprazole in non-erosive reflux disease patients; focused on histologic findings and inflammatory biomarker

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The prospective study on the effect of ilaprazole in non-erosive reflux disease patients; focused on histologic findings and inflammatory biomarker

Directed by Professor Sang Kil Lee

The Master's Thesis submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medical Science.

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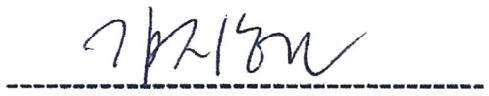

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ABSTRACT

The prospective study on the effect of ilaprazole in non-erosive reflux disease patients; focused on histologic findings and inflammatory biomarker

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INTRODUCTION: Patients with non-erosive reflux disease (NERD) are less responsive to proton pump inhibitors (PPIs) than patients with erosive esophagitis. The aim of this study was to objectively evaluate the effect of a new PPI, ilaprazole, on NERD, assessed using symptom scores, histopathologic findings, and inflammatory biomarkers.

MATERIALS AND METHODS: This prospective study was performed at a single hospital and 20 patients given a clinical diagnosis of NERD were enrolled. All patients underwent 24-hr combined multichannel intraluminal impedance and pH esophageal monitoring (MII-pH) before treatment. Patients were treated with ilaprazole (20 mg) once daily for 4 weeks. The GerdQ questionnaire, histologic findings (basal cell hyperplasia, papillary elongation, dilated intercellular spaces, intraepithelial eosinophils, and intraepithelial T lymphocytes), and inflammatory biomarkers (TNF-α, IL-8, IL-1β, TRPV1, and MCP-1) were used for assessment. Biopsies were obtained from 3 cm above the

esophagogastric junction (EGJ) before and after treatment.

RESULTS**:** Based on MII-pH results, 8 patients (40%) were classified as having functional heartburn. Of the remaining patients, 2 were classified as having true NERD and 10 as having a hypersensitive esophagus. No differences were observed in baseline characteristics among subgroups before treatment, except infiltration of intraepithelial T lymphocytes. After treatment with ilaprazole, all patients showed a statistically significant improvement in GerdQ score ($P < 0.001$). In the histopathologic findings, all parameters showed a tendency to improve and some parameters, such as basal cell hyperplasia ($P = 0.008$), papillary elongation ($P = 0.021$), and infiltration of intraepithelial T lymphocytes ($P = 0.008$), improved significantly. Among cytokines, expression of TNF-α ($P = 0.049$), IL-8 ($P = 0.046$), TRPV1 ($P = 0.045$), and MCP1 ($P = 0.042$) decreased significantly after treatment with ilaprazole.

CONCLUSION**:** Daily ilaprazole (20 mg) is efficacious in improving symptom scores, several histopathologic findings, and some inflammatory biomarkers in patients with clinical NERD, irrespective of subtype.

Key words: gastroesophageal reflux, proton pump inhibitors, ilaprazole

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I. INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic condition that develops when gastric contents flow into the esophagus and cause troublesome symptoms such as heartburn and/or acid regurgitation.[1] Erosive esophagitis and non-erosive reflux disease (NERD) are two subtypes of GERD. Patients with typical reflux symptoms who show no mucosal breaks on conventional endoscopy are defined as having NERD. In clinical practice, NERD is more common than reflux esophagitis. NERD differs in pathophysiology and clinical characteristics from reflux esophagitis. Furthermore, patients with NERD have a lower response rate to proton pump inhibitors (PPIs).[2]

Savarino et al. sub-classified NERD into 3 subtypes: (1) true NERD, with abnormal distal esophageal acid exposure time, (2) hypersensitive esophagus, defined as normal distal esophageal acid exposure time and positive symptom association for either acid and/or non-acid reflux, and (3) functional heartburn with normal distal esophageal acid exposure time and negative symptom

association for acid and nonacid reflux.[3]

The diagnostic value of endoscopic biopsy with histopathologic study is known to be limited; however, histopathologic study has recently attracted renewed attention because the histopathological criteria for recognition of microscopic reflux esophagitis were agreed upon by an international group of expert pathologists.[4, 5] Histologic study can also be used to monitor response after PPI.[6, 7]

PPIs are the most effective drugs for the treatment of GERD. Many PPIs have been developed, including omeprazole, rabeprazole, pantoprazole, lansoprazole, and esomeprazole, and they are widely used for the management of acid-related diseases, such as peptic ulcers and GERD. PPIs are highly recommended for the management of NERD and are thought to provide symptomatic improvement.[8]

Ilaprazole is the latest proton pump inhibitor and is almost equivalent to omeprazole for control of gastric acid secretion.[9] Ilaprazole is also considered to improve symptoms. It has a more prolonged half-life than other PPIs and shows powerful dose-dependent inhibition of symptom. Its safety is similar to that of omeprazole.[10] Although a few studies have been published on the efficacy of ilaprazole for treatment of acid-related diseases,[9, 11, 12] studies on the efficacy of ilaprazole for treatment of NERD are lacking.

Here, we prospectively investigated the efficacy of ilaprazole for treatment of NERD subgroups, as assessed by 24-hr combined multichannel intraluminal impedance and pH esophageal monitoring (MII-pH) using standardized histologic criteria and inflammatory biomarkers.

II. MATERIALS AND METHODS

1. Patients

Eligible patients included adults who had typical reflux symptoms at least twice weekly or a GerdQ score ≥ 8 who were admitted to Severance Hospital, Korea between July 2014 and August 2015. Patients were between the ages of 20 and 80 years. They showed no erosion at the gastroesophageal junction in

esophagogastroduodenoscopy (EGD). All patients provided informed consent to participate in this study.

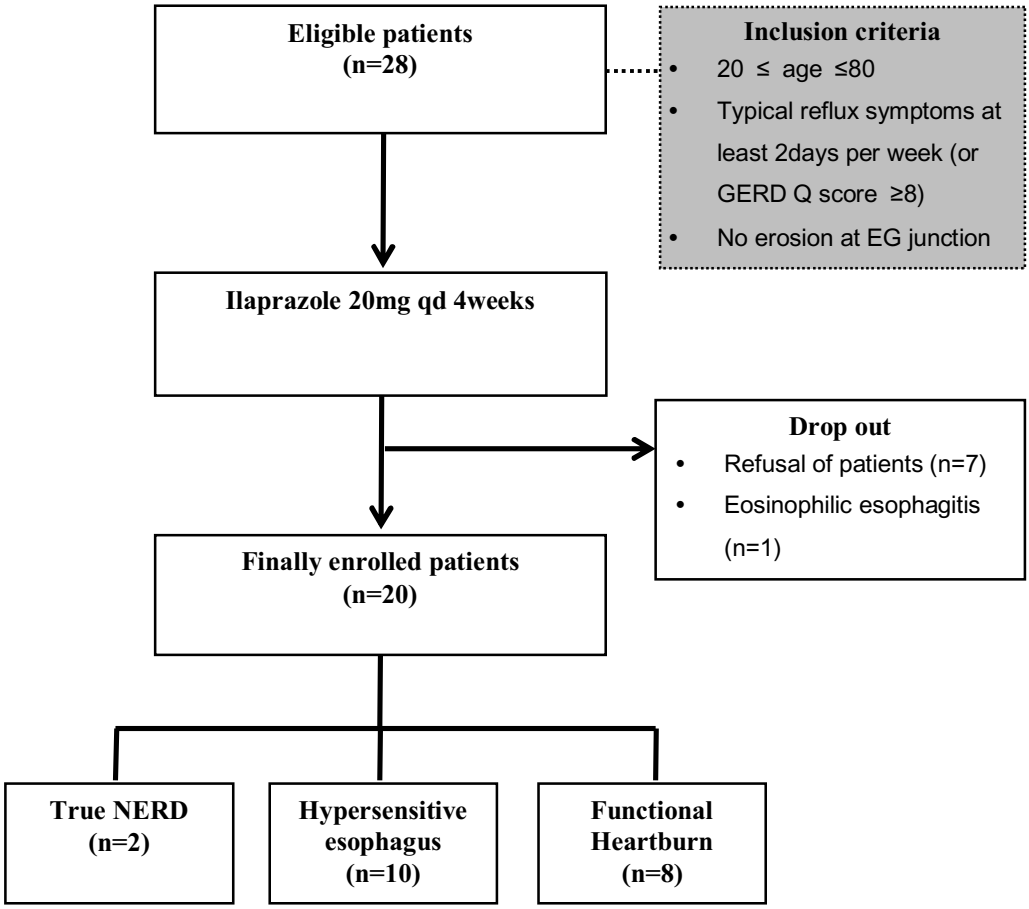
The following exclusion criteria were used: (A) a prior history of documented intolerance of ilaprazole or similar PPIs; or (B) unsuspected alarm symptoms such as weight loss, hematemesis, hematochezia, jaundice, or other significant illness such as a malignancy; or (C) alcoholism or drug addiction; or (D) uncontrolled diabetes, cerebrovascular accident, or diseases requiring an operation in the 3 months prior to enrollment; or (E) any previous esophageal surgery; or (F) malignancy in the gastrointestinal tract within 5 years; or (G) pregnancy; or (H) Zollinger–Ellison syndrome, Barrett's esophagus, primary esophageal motility abnormality, esophageal stricture, duodenal ulcer, gastric ulcer, pancreatitis, absorption disorder, severe cardiovascular disease, severe lung disease in the 3 months prior to enrollment; or (I) a history of prolonged use of medicine such as diazepam, quinidine, diphenylhydantion, mephenytoin, warfarin, anticholinergic, prostaglandin analog, antineoplasatic agent, salicylate, steroid, pro-motility drugs, nonsteroidal anti-inflammatory drugs (NSAIDs); or (J) patients who were registered for other exams within 28 days, or (K) patients who could not undergo a sedated endoscopy.

Potential study participants were screened and enrolled by a research coordinator. Written informed consent was obtained from each patient before enrollment. The study protocol was approved by the Yonsei University College of Medicine Ethics Committee (Institutional Review Board Number: 4-2014-0110) and was registered at ClinicalTrials.gov (NCT02666976).

2. Study design

This study was a single-center, open-label, single-arm, prospective study to objectively evaluate the efficacy of ilaprazole (®Noltec; IL-YANG Pharmaceutical Co., Ltd., Seoul, Korea) for patients with NERD. The study design is shown in Figure 1.

Figure 1. Study design



All subjects were suspected to have NERD and agreed to enroll to our study. Patients who met the inclusion criteria underwent MII-pH. Before MII-pH, the intra-esophageal pressure of all subjects was tested. The results of MII-pH were used to classify patients into three groups: a true NERD group, a group with hypersensitive esophagus, and a functional heartburn group. Patients completed GerdQ questionnaires regarding their gastroesophageal reflux symptoms. They were treated with ilaprazole (20 mg) once daily for 4 weeks. After taking ilaprazole, all subjects underwent EGD and complete the GerdQ questionnaire again. PPIs, H2RAs, prokinetic agents, bismuth preparations, antacids, and

other substances that could influence the relief of symptoms related to acid secretion were not permitted during the study. We aimed to enroll 37 patients, but the study was concluded prematurely owing to the difficulty of subject enrollment and the prolonged study period.

3. Endoscopy and histopathologic evaluation

The EG junction was defined as the most proximal extent of the gastric folds during endoscopy. In endoscopic examinations before and after treatment, 3 tissue samples were obtained from 3 cm above the EG junction for histologic evaluation. The biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. Slides stained with hematoxylin-eosin were used for general evaluation. Another 3 tissue samples were biopsied from the same location for analysis of inflammatory biomarkers before and after treatment. The samples were immediately stored in RNAlater (Ambion Inc., Austin, TX), and maintained at -80℃ until measurement of messenger RNA (mRNA). Total mRNA was extracted according to the manufacturer's instructions using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). Contaminated DNA was removed by treatment with RNase-free DNase (Invitrogen). For complementary DNA synthesis, total RNA was reverse transcribed using SuperscriptTMII (Invitrogen) following the manufacturer's protocol. Quantitative PCR (qPCR) was performed using iQ SYBR Green Supermix (Applied Biosystems Inc., Carlsbad, CA, USA) and conducted on a Roche LightCycler480 Real-Time PCR System. The target sequences for qPCR were as follows: TNF-α (F-5′CAGCCTCTTCTCCTTCCTGAT3′; R-5′GCCAGAGGGCTGATTAGAGA3′), IL-8 (F-5′GCAGCCTTCCTGATTTCTGCAGCTC3′; R-5′ACTTCTCCACAACCCTCTGCACCCA3′), IL-1β (F-5′CCAGCTACGAATCTCGGACCACC3′; R-5′TTAGGAAGACACAAATTGCATGGTGAAGTCAGT3′), TRPV1

(F-5′GAGTTTCAGGCAGACACTGGAA3′;
R-5′CTATCTCGAGCACTTGCCTCTCT3′), MCP-1
(F-5′GATCTCAGTGCAGAGGCTCG3′;
R-5′TGCTTGTCCAGGTGGTCCAT3′), GAPDH
(F-5′CCGGGAAACTGTGGCGTGATGG3′;
R-5′AGGTGGAGGAGTGGGTGTCGCTGTT3′). GAPDH was used as an endogenous control and the Ct value was normalized to GAPDH using the 2-ΔΔCt method. Only reliable qRT-PCR data points were used to analyze the effect of the drug.

4. Manometric study

We used an eight-channel, water-perfused esophageal manometry catheter (MUI Scientific Company, Ontario, Canada) and a water-perfused, low-compliance perfusion system (Synetics Medical Co., Stockholm, Sweden). The manometric analysis was performed before the MII-pH study. During the esophageal manometry study, we evaluated variable parameters such as the resting LES pressure, the length of LES, the amplitude of pressure waves, and the duration of pressure waves.

5. 24hr-combined multichannel intraluminal impedance and pH esophageal monitoring (MII-pH)

MII-pH was performed before administration of ilaprazole, using an ambulatory multichannel intraluminal impedance (MII) and pH monitoring system (Sleuth; Sandhill Scientific, Inc.
Highland Ranch, CO). The DeMeester score, distal esophageal acid exposure time (AET), total number of reflux episodes, symptom association probability (SAP), and the symptom index (SI) were determined. The distal esophageal AET was defined as the total time with a pH < 4, divided by the total monitoring time. A percent time < 4.2% with pH < 4 over 24 hours was referred

to as normal.[3] Pathologic acid reflux was defined as distal esophageal acid exposure percent time > 4.2%. The SAP was calculated for acid and nonacid reflux using a custom-made Excel macro function. The SAP was considered to be positive when ≥ 95%.[13] The SI was calculated using the Bioview analysis software (Sandhill Scientific, Inc.). It was defined as the number of symptoms associated with reflux divided by the total number of symptoms. The SI was considered to be positive when ≥ 50%.[14] We defined a positive symptom association for reflux as either a positive SAP or a positive SI.

Patients were diagnosed as having true NERD when the distal esophageal acid exposure percent time was > 4.2% or the DeMeester score was > 14.7%, based on the MII-pH results. Patients with a normal distal esophageal acid exposure time and a positive symptom association for either acid and/or non-acid reflux were classified as patients with a hypersensitive esophagus. Patients were diagnosed with functional heartburn when they showed a normal distal esophageal acid exposure time and negative symptom association for acid and nonacid reflux. Patients with a hypersensitive esophagus could include patients with an acid, weak acid, and non-acid sensitive esophagus.

6. Outcome measures

The aim of this study was to demonstrate the efficacy of ilaprazole for treatment of patients with NERD. Our primary endpoint was changes in symptoms (GerdQ score) after ilaprazole. All patients complete the GerdQ questionnaire before and after taking ilaprazole, and it was utilized to assess improvement of symptoms after taking the medicine. The GerdQ questionnaire is composed of 6 items and can be used as a diagnostic tool for GERD. In a previous study, it was shown to have similar diagnostic power to a routine symptom-based diagnosis made by a clinician, and a cut-off score ≥ 8 showed a sensitivity of 78% and a specificity of 64%.[15, 16] It has also been used as a tool for evaluating the PPI response in GERD patients.[17]

Our secondary endpoint included changes in histopathological findings and inflammatory biomarkers on tissue analysis after treatment. Histologic findings include basal cell hyperplasia, papillary elongation, dilated intercellular spaces, intraepithelial eosinophils, and intraepithelial T lymphocytes. All specimens were assessed by a single histologist (H.K.). Variable histologic findings were assessed by light microscopy. To classify the severity of the histologic findings, we used the histologic criteria and severity score (score range, 0–2) set by the Esohisto project.[18, 19] Basal cell layer thickness and papillary length were measured in micrometers. Both proportions of total epithelial thickness were calculated. The severity of basal cell layer hyperplasia was graded as 0 (< 15%), 1 (15–30%), or 2 (> 30%). The severity of papillary elongation was expressed as 0 (< 50%), 1 (50–75%), or 2 (> 75%). We evaluated irregular round dilations or diffuse widening of the intercellular space, which can be graded as 9 (absent), 1 (small; diameter < 1 lymphocyte), or 2 (large; diameter ≥ 1 lymphocyte). Intraepithelial eosinophils and T lymphocytes were counted in the most affected high-power field. The severity of intraepithelial eosinophils was defined as 0 (absent), 1 (1–2 cells), or 2 (> 2 cells). Intraepithelial T lymphocytes were scored as 0 (0–9 cells), 1 (10–30 cells), or 2 (> 30 cells). Inflammatory biomarkers (TNF-α, IL-8, IL-1β, TRPV1, and MCP-1) were also assessed by RT-PCR.

7. Statistical analysis

Calculations were performed using the SPSS statistical software version 18.0 (SPSS, Chicago, IL, USA). Continuous variables are presented as mean ± standard deviation (SD) and discrete variables are expressed as numbers and percentages. The baseline characteristics of the patients in the three groups were compared using the chi-squared test, or Fisher's exact test, one-way ANOVA, and the Kruskal–Wallis test. Paired comparisons of parameters before and after

taking ilaprazole were performed using a paired t-test or a Wilcoxon signed-rank test. P-values < 0.05 were considered statistically significant. Results are presented as odds ratios (ORs), 95% confidence intervals, and P-values. $P \leq 0.05$ was considered statistically significant.

Using a significance level of 5% and a statistical power of 90% with a two-sided test, a sample size of 37 patients was required for our treatment group, assuming a 30% drop-out rate.

III. RESULTS

1. Baseline characteristics of patients

Twenty-eight individuals participated in our study. Of these subjects, 8 patients were excluded from the study; 7 patients discontinued due to improvement of symptoms or unexpected patient refusal, 1 patient was diagnosed with eosinophilic esophagitis based on the histological results. Thus, 20 patients completed the treatment and were analyzed. Based on MII-pH analysis, 8 patients (40%) were classified as having functional heartburn. The remaining patients were defined as having NERD (60%), including 2 with true NERD and 10 with a hypersensitive esophagus. The 10 patients with a hypersensitive esophagus included 5 with an acid-sensitive esophagus and 5 with a weak acid-sensitive esophagus. None of the patients were diagnosed as having a non-acid-sensitive esophagus. Baseline patients' characteristics are shown in Table 1 and Table 2

Table 1. Baseline clinical parameters and inflammatory biomarkers among subgroups

Parameters	True NERD	Hypersensitive esophagus	Functional Heartburn	Total	p-value
Mean age(years)	69.0±12.7	59.2.0±10.7	57.4± 6.1	59.5 ± 9.4	0.307
Male(%)	1(50.0)	2(20.0)	1(12.5)	4(20.0)	0.74
GERD-Q score	11.0±1.4	11.1±1.9	11.1±2.6	11.1±2.1	0.997
AET(acid exposure time)(%)	10.0 ± 4.5	1.1 ± 1.2	0.5 ± 0.7	1.73±3.2	**<0.001**
Total number of reflux episodes	49.0 ± 46.	32.0 ± 15.3	42.4 ± 33.7	37.8 ± 26.1	0.601
Acid refluxes	37.5 ± 34.6	14.9 ± 12.1	13.3 ± 8.3	16.5 14.5	0.088
TNF-α (fold change to GAPDH, x10^4) (n=16) *	0.66 ± 0. 61 (n=2)	0.24 ± 4.77 (n=8)	3.53 ± 4.39 (n=6)	2.61 ± 4.39 (n=16)	0.742
IL-8 (fold change to GAPDH, x10^4) (n=19) *	2.50 ± 0.40 (n=2)	4.45 ± 6.94 (n=10)	6.73 ± 11.09 (n=7)	5.09 ± 8.19 (n=19)	0.783
IL-1β (fold change to GAPDH, x10^4) (n=19) *	0. 27 ± 0.15 (n=2)	0.34 ± 0.12 (n=9)	0.38 ± 0.23 (n=8)	0.35 ± 0.17 (n=19)	0.724
TRPV1(fold change to GAPDH, x10^4) (n=18) *	0. 24 ± 0.14 (n=2)	0.63 ± 0.89 (n=9)	1.02 ± 0.90(n=7)	0.74 ± 0.85 (n=18)	0.475
MCP-1(fold change to GAPDH, x10^4) (n=14) *	9.19 ± 3.17(n=2)	15.09 ± 9.7 (n=9)	19.62 ± 12.88 (n=6)	16.00 ± 10.55 (n=17)	0.477

* TNF-α, IL-8, TRPV1, MCP-1 and GAPDH were measured by qRT-PCR. The

GAPDH was used as the endogenous control. The Ct value of TNF-α, IL-8, TRPV1 and MCP-1 were normalized to GAPDH using 2-ΔΔCt method. Fold change of TNF-α, IL-8, TRPV1 and MCP-1 to GAPDH was amplied by 10^4 fold for convenience. Some of samples were not qualified to do qRT-PCR, only reliable data values of qRT-PCR were used to analyze (TNF-α;16, IL-8;18, IL-1β;19, TRPV1;18, MCP-1;14)

Table 2. Baseline histopathological parameters among subgroups

Parameters	severity score	True NERD(N=2)	Hypersensitive esophagus(N=10)	functional heartburn(N=8)	p-value
Basal cell Hyperplasia	0	1(50.0%)	3(30.0%)	2(25.0%)	0.656
	1	0(0.0%)	5(50.0%)	2(25.0%)	
	2	1(50.0%)	2(20.0%)	4(50%)	
papillary elongation	0	0(0.0%)	3(30.0%)	2(25%)	0.098
	1	0(0.0%)	6(60.0%)	2(25%)	
	2	2(100.0%)	1(10.0%)	4(50%)	
Dilated intercellular spaces	0	2(100.0%)	7(70.0%)	4(50%)	0.391
	1	0(0.0%)	3(30.0%)	4(50%)	
	2	0(0.0%)	8(80.0%)	0(0.0%)	
Infiltration of intraepithelial eosinophils	0	2(100.0%)	2(20.0%)	8(100.0%)	0.348
	1	0(0.0%)	10(100.0%)	0(0.0%)	
	2	0(0.0%)	0(0.0%)	0(0.0%)	
Infiltration of intraepithelial T lymphocyte	0	0(0.0%)	0(0.0%)	1(12.5%)	**0.03***
	1	1(50.0%)	6(60.0%)	5(62.5%)	
	2	1(50.0%)	4(40.0%)	2(25%)	

*Hypersensitive esophagus group versus functional heartburn group, p=0.043

The mean age of patients was 59.5 ± 9.4 years, and patients included 16 females and 4 males (80% and 20%, respectively; ratio = 4:1). The mean GerdQ score before treatment was 11.1 ± 2.1. All groups were similar with regard to baseline characteristics.

Among the findings of MII-pH analysis, baseline AET was significantly higher in patients with true NERD than those with hypertensive esophagus and functional heartburn (P < 0.001, table 1). No differences in baseline inflammatory biomarkers were observed among subtypes (Table 1).

Among the pre-treatment histological findings, basal cell hyperplasia, papillary elongation, dilated intercellular spaces, and infiltration of intraepithelial eosinophils did not differ among the subgroups. The infiltration of intraepithelial T lymphocytes were higher in the hypersensitive esophagus group than the functional heartburn group (P = 0.03). No abnormalities or differences among the subtypes were observed among the manometric parameters.

2. Efficacy of ilaprazole on Gerd Q, histology and inflammatory cytokines

After treatment with ilaprazole, patients' GerdQ scores were significantly reduced (P < 0.001, Table 3).

Table 3. Comparison of clinicopathological parameters between before and after treatment

Parameters	severity score	Before treatment	After treatment	p-value
GERD-Q score		11.1±2.1	3.2±3.0	**<0.001**
Basal cell Hyperplasia	0	6(30%)	13(65%)	**0.008**
	1	7(35%)	6(30%)	

	2	7(35%)	1(5%)	
Papillary elongation	0	5(25%)	11(55%)	**0.021**
	1	8(40%)	7(35%)	
	2	7(35%)	2(10%)	
Dilated intercellular spaces	0	13(65%)	17(85%)	0.102
	1	7(35%)	3(15%)	
	2	0(0%)	0(0%)	
Infiltration of intraepithelial eosinophils	0	18(90%)	19(95%)	0.317
	1	2(10%)	1(5%)	
	2	0(0%)	0(0%)	
Infiltration of intraepithelial neutrophils	0	20(100%)	20(100%)	1.000
	1	0(0%)	0(0%)	
	2	0(0%)	0(0%)	
Infiltration of intraepithelial T lymphocyte	0	7(35%)	15(75%)	**0.008**
	1	10(50%)	5(25%)	
	2	3(15%)	0(0%)	

All enrolled patients showed an improved GerdQ score after ilaprazole treatment. The histopathologic findings showed a decreasing tendency, with score for all parameters decreasing; some findings, such as basal cell hyperplasia (P = 0.008), papillary elongation (P = 0.021), and infiltration of intraepithelial T lymphocytes (P = 0.008) were improved significantly (Figure 2).

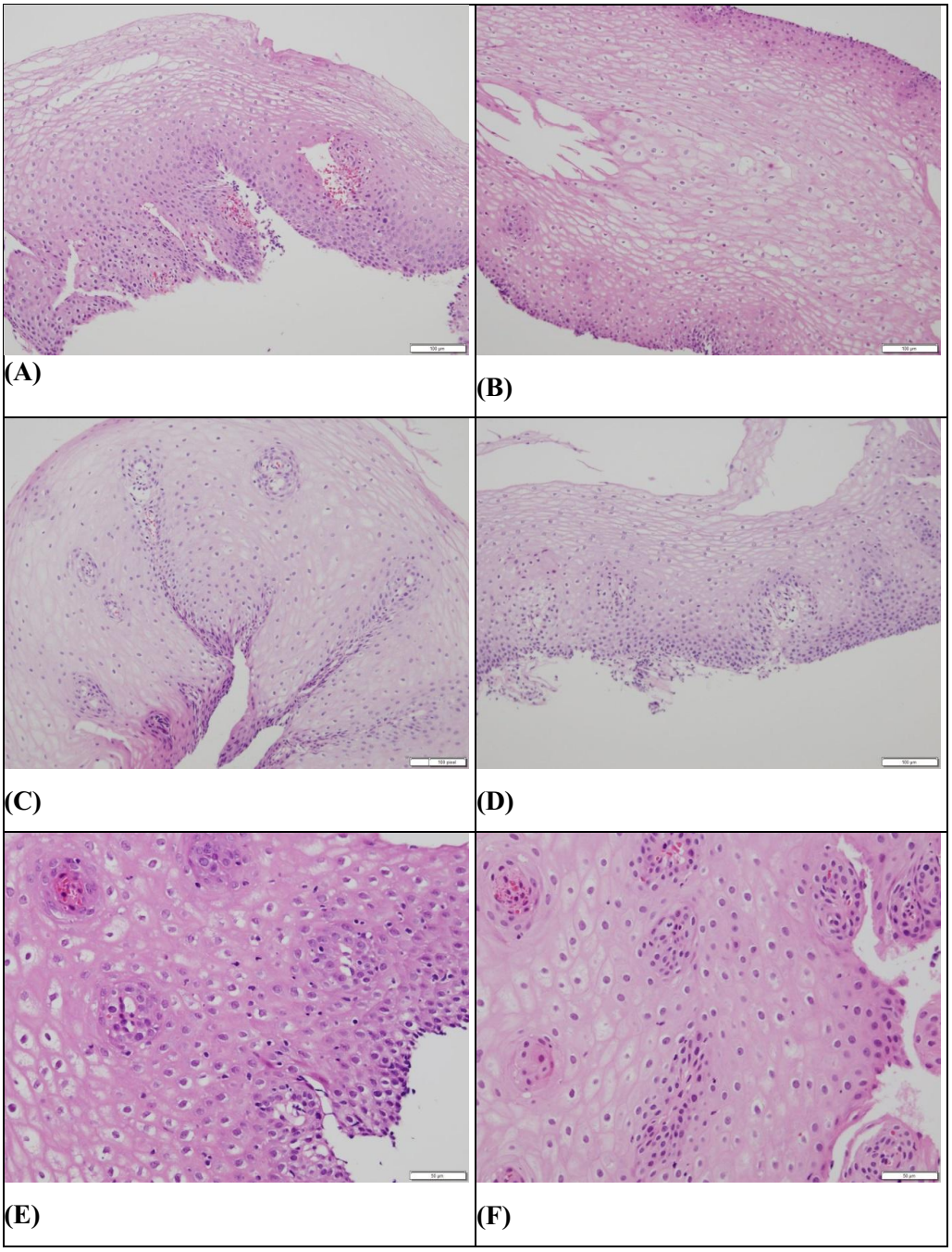


Figure 2. Several significant changes of histologic finding after taking ilaprazole. Before and after treatment, several histologic findings were

improved. The thickness of basal layer was observed from 40%(A) to 10%(B). The length of papillae was improved from 80%(C) to 40%(D) of total epithelial thickness . Infiltration of T lymphocyte was counted from 16(E) to 4(F).

The degree of basal cell hyperplasia was reduced significantly in the hypersensitive esophagus group (P = 0.034) and infiltration of intraepithelial T lymphocytes was improved significantly in the functional heartburn group (P = 0.034).

Among the various cytokines examined, expression of TNF-α (P = 0.049), IL-8 (P = 0.046), TRPV1 (P = 0.045), and MCP1 (P = 0.042) were decreased significantly after treatment with ilaprazole (Figure 3).

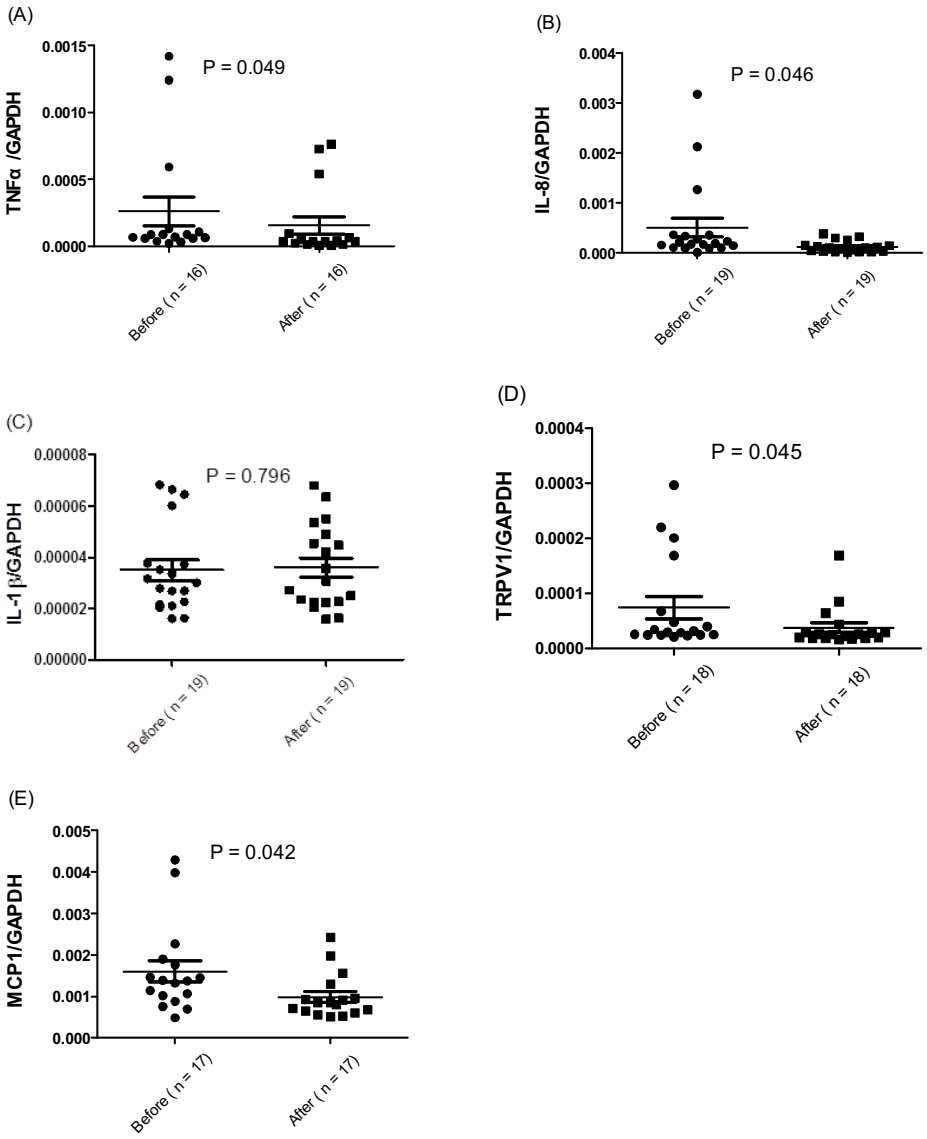


Figure3. Comparison of inflammatory biomarkers between before and after treatment

IV. DISCUSSION

This is the first prospective study to show the effect of ilaprazole on patients with NERD, using not only subjective measures, such as GerdQ scores, but also objective methods, including standardized histologic parameters and inflammatory markers. Based on GerdQ scores, 90% of patients showed an improvement following treatment with ilaprazole. Ilaprazole significantly improved 4 of the 5 inflammatory markers (TNF-α, IL-8, TRPV1, and MCP-1), as measured by qRT-PCR, and also significantly improved histologic parameters such as basal cell hyperplasia, papillary elongation, and infiltration of intraepithelial T lymphocytes. We showed that ilaprazole is effective for treatment of patients with NERD in terms of symptoms, histological findings, and inflammation.

We attempted to measure symptoms objectively, using the GerdQ questionnaire as a symptom rating scale. The GerdQ score was developed as a diagnostic tool for use with primary care patients with GERD. A GerdQ score of 8 has been determined to be an appropriate cutoff value in the Japanese population; we also selected this criterion.[20] All of the patients enrolled had a GerdQ score ≥ 8. After treatment with ilaprazole, 18 of 20 patients showed a GerdQ score < 8. Thus, 90% of the enrolled patients could be judged to be recovered, based on their GerdQ scores. It is known to be difficult to evaluate the response to PPI objectively, using only the severity of symptoms. Several studies have been published that measured the objective efficacy of PPI in reflux disease. [21] [22]

In our study, we adapted the MII-pH to subdivide the patients into three groups. The proportion of patients with true NERD/hypersensitive esophagus/functional heartburn was 10%/50%/40%. The ratio of patients with functional heartburn correlated well with a previous report, but the number of patients with true NERD was somewhat lower than in previous studies.[23-25] All

of the patients with true NERD showed symptomatic improvement based on GerdQ score, histologic parameter score, and inflammatory markers. In a recent study, patients with true NERD showed a response to PPI similar to that of patients with ERD.[26] The effect of ilaprazole seems to be strong for patients with true NERD compared to those with a hypersensitive esophagus and functional heartburn; however, the number of patients with true NERD included in our study was too small to confirm this finding. Despite our best efforts to enroll patients with NERD who showed typical symptoms of GERD, only 10% of patients were diagnosed with true NERD.

In our study, most of the patients with functional heartburn showed symptomatic improvement following treatment with ilaprazole. In functional heartburn, PPI responders are known to have a higher acid exposure time, more frequent reflux events, and more acid reflux compared with PPI non-responders. The impedance pH features of patients with hypersensitive esophagus were similar to those of PPI responders with functional heartburn.[27] In our study, no differences were observed in impedance test results, such as AET, number of reflux events, and acid reflux, between the hypersensitive esophagus and functional heartburn groups. We hypothesize that the patients in the functional heartburn group might have been primarily composed of PPI responders; this could have induced the high rate of response to ilaprazole observed among the functional heartburn group in our study.

Recently, microscopic changes in GERD, including NERD and functional heartburn, have received attention as diagnostic and monitoring tools. Pathologic reflux in the esophagus results in injury to epithelial cells of the mucosa. It can lead to promotion of cell turnover and basal cell hyperplasia. Hyperemia of the capillaries presents as papillary elongation.[28] Infiltration of the epithelium by inflammatory cells could be used as a parameter for diagnosis of GERD. Infiltration of lymphocytes appeared to be more frequent than infiltration with eosinophilic or neutrophilic granulocytes. Infiltration of

intraepithelial lymphocytes is known to be observed in 42% of patients with NERD. [29] A previous study showed significant histopathological differences between NERD and functional heartburn. These histopathological findings generally include the degree of basal cell hyperplasia, presence of papillary elongation, dilation of intercellular spaces, and infiltration of inflammatory cells.[30] According to our data, infiltration of intraepithelial T cells only differed between two subgroups. This discrepancy with previous reports might also be caused by a higher proportion of PPI responders in the functional heartburn group and the small sample size.

Basal cell hyperplasia, papillary elongation, and infiltration of T lymphocytes were improved after treatment with ilaprazole in all patients. All of these histologic markers have been recognized as evidence of reflux disease.[31] As studied earlier, whether histologic changes revert to normal can be used to judge the efficacy of PPIs such as esomezole.[22] Ilaprazole also improved these microscopic findings in the present study. In the subgroup analysis, the hypersensitive esophagus group differed from the functional heartburn group. Among the three parameters that showed improvement after treatment with ilaprazole, basal cell hyperplasia and papillary elongation were improved in the hypersensitive esophagus group; however, only infiltration of T lymphocytes was improved in the functional heartburn group. This may imply differences of undisclosed pathophysiology between the hypersensitive esophagus and functional heartburn groups, even if the functional heartburn group included a higher proportion of PPI responders.

Among the histologic changes that occur with GERD, dilatation of intercellular space (DIS) is known as an early marker of tissue injury in gastro-esophageal reflux disease and non-erosive reflux disease. DIS can be experimentally induced by acidic or weakly acidic reflux.[32] A previous study demonstrated that DIS was very well correlated with acid exposure time of the distal esophagus in NERD and that patients with abnormal AET are more likely to show DIS than

those with normal AET.[33] In our study, DIS improved after treatment with ilaprazole, but not significantly; thus, DIS was not a better indicator of the reaction to ilaprazole than other histological changes. This might have been caused by the high proportion of patients with functional heartburn in our study population, because DIS was recently reported to have diminished diagnostic value in functional heartburn.[34]

Recent studies have demonstrated that some immune and inflammatory mechanisms are mediated by specific cytokines or chemokines in GERD.[6] IL-8 is a neutrophil chemotactic factor that plays a crucial role in inducing inflammation, and is a representative chemokine in the pathogenesis of NERD.[35] IL-1β also appears to play an important role including inflammation and fibrosis, and is known to be related to NERD.[36] TNF-α induced transcription of oncogenes, such as *c-myc*, and its epithelial expression was increased in the progress of Barrett's esophagus to adenocarcinoma. IL-1 β and TNF- α may be related to the Barrett's esophagus-dysplasia-carcinoma sequence.[6] MCP-1 attracts monocytes and lymphocytes and was higher in patients with reflux esophagitis than healthy controls. [6]

Transient receptor potential vanilloid-1 (TRPV1) has been implicated in the mechanism of acid-induced inflammation in GERD.[37] These markers were tested for use as tools in determining the efficacy of drugs in treating NERD. The expression of IL-8, IL-1β, and MCP-1 was decreased by treatment with PPIs such as lansoprazole.[38-40] In our study, all inflammatory biomarkers except IL-1 β decreased after treatment with ilaprazole, with moderate amplitudes.

This study has some limitations. First, the number of subjects was small because the study was ended due to poor enrollment. However, the subgroup proportions were similar to those reported in studies of large populations. This means there will be less bias, even though the number of patients with true NERD was only 2. Although we could not judge the reliability of the results in the true NERD group, we could confirm the efficacy of ilaprazole in all patients

with NERD and observe the approximate progress of treatment, including in the functional heartburn group. Second, we had no control group. To improve confidence, a comparison study between the efficacy of ilaprazole and that of other PPIs is required. Third, we did not consider the placebo effect of ilaprazole itself in our results. Ilaprazole is expected to show a placebo effect on symptomatic responses, similar to other PPIs.[41] We introduced a highly objective method, including histological exams and use of biomarkers, in order to exclude a possible placebo effect.

V. CONCLUSION

Despite these limitations, we showed that ilaprazole was effective in treatment of patients with NERD in terms of symptoms, histology, and inflammation. We identified differences in histologic features between the hypersensitive esophagus and functional heartburn groups, although the efficacy of ilaprazole was the same in both subgroups. These findings imply that ilaprazole might act on hypersensitive esophagus and functional heartburn via an unknown mechanism. Although further study, including inclusion of a larger number of patients with NERD, is needed to validate our findings, our finding suggest that ilaprazole might be effective for treatment of NERD, including functional heartburn.

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ABSTRACT(IN KOREAN)

비미란성 위식도 역류질환 환자에서 일라프라졸의 치료 효과 : 조직학적 소견과 염증 인자의 변화를 중심으로

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서론 : 비미란성 위식도 역류질환 환자는 미란성 위식도 역류질환 환자에 비하여 프로톤 펌프 억제제에 잘 반응하지 않는다고 알려져 있다. 본 연구의 목적은 새로운 프로톤 펌프 억제제인 일라프라졸이 비미란성 위식도 역류질환 환자의 치료에 미치는 영향을 증상 점수, 조직학적 소견 및 염증인자의 변화를 중심으로 살펴보는 데 있다.

재료 및 방법 : 임상적으로 비미란성 위식도역류질환으로 진단된 20명의 환자를 대상으로 단일기관에서 전향적 연구로 진행되었다. 대상환자들은 24시간 다채널 강내 임피던스와 pH 검사를 시행한 후 4주간 하루 한번 일라프라졸 20밀리그램을 투여하였다. 치료 전후 증상 점수 분석을 위하여 GERD Q 설문지를 작성하였고, 위식도 접합부 3 센치미터 상방에서 조직검사를 시행하여 조직학적 소견 및 염증인자를 분석하였다.

결과 : 24시간 다채널 강내 임피던스와 pH 검사를 통해 8명의

환자는 기능성 가슴쓰림 환자로 분류하였고 2명은 진성 비미란성 위식도 역류질환으로, 10명은 과감작 식도 환자로 진단하였다. 상피내 T 림프구 침착을 제외하면 이들간의 조직학적 소견의 차이는 없었다. 일라프라졸 투여후 GERD Q 점수에서 의미있는 호전을 보였으며, 기저세포 증식, 유두상 연장 및 상피내 T 림프구 침착 등의 조직학적 소견은 호전되는 소견 경향을 보였다. 특히 과감작 식도환자군에서는 기저세포 증식이, 기능성 가슴쓰림 환자군에서는 상피내 T 림프구 침착이 유의미한 감소를 보였다. 염증인자 분석에서는 TNF-α, IL-8, TRPV1 및 MCP1이 일라프라졸 투여후 유의미한 감소를 보였다.

결론 : 비미란성 위식도역류질환에서 일라프라졸은 증상 점수 및 몇몇 조직학적 소견과 염증 인자를 개선시킨다. 과감작 식도 환자 및 기능성 가슴쓰림 환자에서의 몇몇 조직학적 반응은 다르게 나타날 수 있으나, 두 그룹 모두에서 일라프라졸은 효과적인 치료제임을 객관적으로 보여주었다.

핵심되는 말 : 위식도 역류, 프로톤 펌프 억제제, 일라프라졸