ORIGINAL PAPER

DOI: http://dx.doi.org/10.15403/jgld-5859

The Mucosal Protection in the Treatment of Erosive Reflux Esophagitis: Mechanisms for Restoring Epithelial Permeability. A Randomized Clinical Trial

Dmitry S. Bordin[1,2,3], Maria A. Livzan[4], Sergei I. Mozgovoi[4], Olga V. Gaus[4], Irina V. Lapteva[4]

1) Loginov Moscow Clinical Scientific Center, Moscow;
2) Russian University of Medicine, Moscow;
3) Tver State Medical University, Tver;
4) Omsk State Medical University, Omsk, Russia

Address for correspondence:
Maria A. Livzan
Omsk State Medical University, 12 Lenina Str., Omsk, Russia, 644099.
mlivzan@yandex.ru

Received: 23.08.2024
Accepted: 13.10.2024

ABSTRACT

Background & Aims: Gastroesophageal reflux disease (GERD) is widespread in the population and is characterized by the risk of developing Barrett's esophagus and associated adenocarcinoma. Key factors in the progression of the disease are not only the frequency and duration of reflux episodes, but also the resistance of the esophageal mucosa to aggressive reflux molecules. Assessment of the state of tight junction proteins, the rate of their recovery under the influence of various treatment regimens is an urgent task for choosing optimal approaches to curing patients with GERD. The purpose of the study was to evaluate the efficacy and safety of the combination therapy with a proton pump inhibitor (PPI) and a topical mucosal protective agent (MPA) to relieve GERD symptoms and achieve a complete remission of reflux esophagitis faster.

Methods: 60 patients (38 men and 22 women with an average age 41.5 years) with GERD duration of 5 years, took part in an open randomized prospective clinical study. Participants were randomized in a 1:1 ratio to be divided into the main group taking MPA and PPI and a comparison group taking a PPI only. The duration of therapy was 4 weeks. Before the treatment onset and after the end of treatment, the occurrence and severity of GERD symptoms were assessed, an endoscopic examination was performed and biopsy specimens were taken from the edge of the erosive area and the area of unchanged esophageal mucosa. The severity of histological signs of esophagitis, the expression of tight junction proteins and the proliferation marker Ki-67 were assessed.

Results: A more effective relief of GERD symptoms was documented when using combination therapy PPIs and MPAs. A more pronounced reduction in macroscopic changes was found in patients with erosive esophagitis taking PPIs and MPAs. The use of MPAs in addition to PPIs made it possible to achieve the primary and secondary endpoints more often, which suggested a high efficiency of the drug in combination with PPIs in the treatment of erosive esophagitis.

Conclusions: An individualized approach based on the combination therapy of PPIs and MPAs can improve the effectiveness of treatment in achieving clinical, endoscopic and, more importantly, histological remission in a patient with erosive GERD.

Key words: gastroesophageal reflux disease – erosive esophagitis – esophagus mucosal resistance – epithelial permeability – esophageal mucosal protection.

Abbreviations: GERD: gastroesophageal disease; MPA: mucosal protective agent; PPI: proton pump inhibitor.

INTRODUCTION

Gastroesophageal reflux disease (GERD) occupies a leading position in terms of prevalence among acid-dependent diseases and is defined as a condition that develops when gastric contents enter the esophagus with the appearance of characteristic symptoms and/or complications [1-3]. The key factors determining the progression of the disease are the occurrence frequency and the duration reflux [3]. In addition to aggression factors, it is necessary to take into account the protective properties' of esophageal mucosa and its resistance to the effects of aggressive refluxate molecules (hydrochloric acid, pepsin, bile acids and lysolecithin) [4].

Proton pump inhibitors (PPIs) - first-line drugs for GERD treatment can significantly reduce the aggressiveness of refluxate [5, 6]. However, in real clinical practice, PPI monotherapy has limited efficacy and a certain group of patients with GERD requires a combination of PPIs with drugs that normalize motility and restore resistance of the esophageal mucosa [6].

Alfazoxx (Alfasigma, S.p.A., Italy) was used as a local mucosal protective agent (MPA). It is a medical device, consisting of a mixture (in a ratio of 1:2.5) of low molecular weight (80-100 kDa) hyaluronic acid and low molecular weight (10-20 kDa) chondroitin sulfate, which are dissolved in a bioadhesive carrier (poloxamer 407), and together form a macromolecular complex that promotes the restoration of the esophageal mucosa and acts as a mechanical barrier, uniformly enveloping the esophageal wall and preventing contact with damaging refluxate molecules [7-9].

In the presented open prospective randomized parallel study the effectiveness of the combination therapy with PPI and MPA was studied for the relief of the GERD symptoms and the reduction stigmas of reflux esophagitis.

METHODS

Participants

The studies included patients aged 18–70 years with grade C and grade D erosive esophagitis according to the Los Angeles classification [1].

Patients with Barrett's esophagus, ulcers, peptic strictures, bleeding, malignancies of the esophagus or stomach were excluded from the study. Patients with viral hepatitis or tuberculosis, severe somatic diseases were excluded from the study. Pregnant and breastfeeding women were not allowed to participate in the study.

Study Design

This open randomized prospective clinical study protocol complies with the principles of the Declaration of Helsinki and was approved by the local Ethics Committee of the Omsk State Medical University (registration number AAAA-A20-120040790011-1).

All patients eligible to participate in the study gave written informed consent, after which they were asked to undergo a preparation period of 14 days when any (prescription or over-the-counter) antisecretory drug therapy was discontinued (visit 1). The only allowed drugs were antacids or alginate-containing drugs for the relief of the GERD symptoms.

At visit 2, patients were randomized in a 1:1 ratio. The main group received therapy with a standard dose of PPI pantoprazole 30 minutes before breakfast and Alfasoxx at a dose of 10 ml (1 sachet) three times a day after meals and the fourth sachet 30 minutes before bedtime. The comparison group received standard PPI therapy. The duration of therapy in each group was 4 weeks. Data on study compliance and adverse events were collected via telephone contact. Before (visit 2) and after (visit 3) the course of therapy, the frequency and severity of GERD symptoms were assessed using the Likert scale. After 4 weeks from the start of therapy (visit 3), all patients underwent endoscopic examination. In previously randomly selected patients, biopsy specimens were taken from the edge of the erosive area and the area of the unchanged mucosa of the esophagus at a distance of two centimeters from the lower esophageal sphincter for repeated morphological and immunohistochemical studies. Patient compliance was defined as the percentage of the used drug, obtained by counting returned drugs at visit 3. Treatment adherence of 80-100% was considered acceptable. Safety and tolerability were assessed by recording all adverse or unexpected symptoms that could be related to the drugs used in the study. The study design is shown in Fig. 1.

GERD Clinical Manifestations

Complaints were evaluated using the Likert scale. The patient was asked to rate the frequency and severity of each

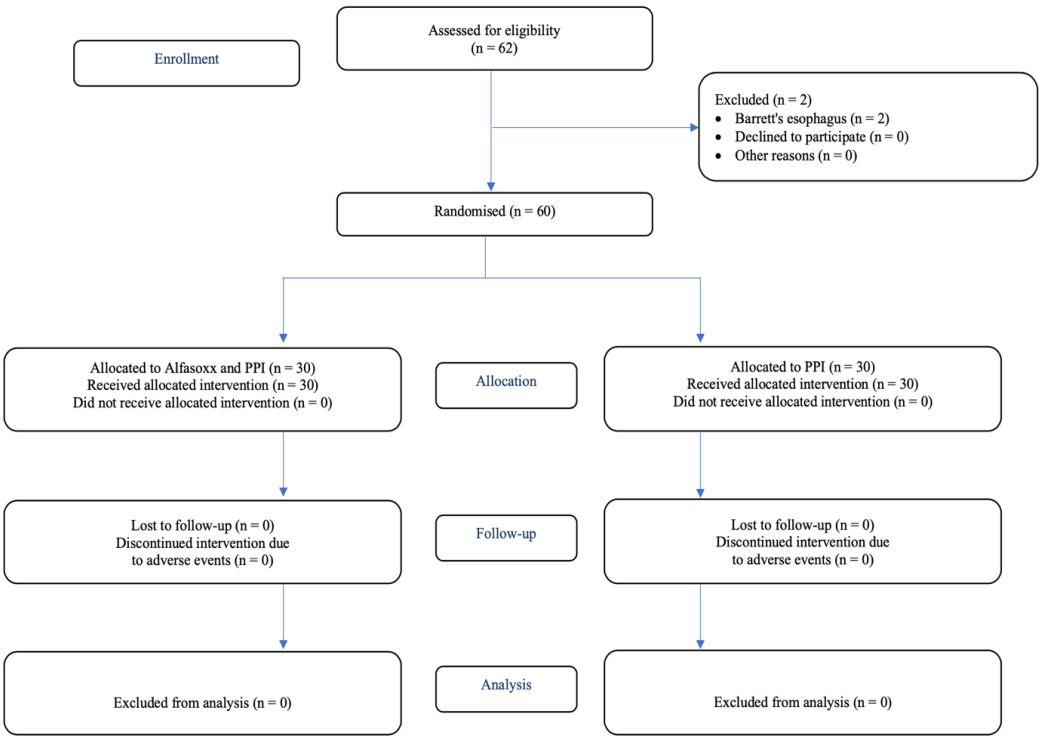

Fig. 1. Design of the study.

of the GERD symptoms (heartburn, chest pain, reflux/sour regurgitation), eructation, dysphagia, odynophagia).

Endoscopy

At the stage of inclusion (visit 1) and after the end of the therapy course (visit 3), all patients underwent a narrow band endoscopy (NBI), magnifying endoscopy (ZOOM) and an iScan. In each group of 15 randomized patients, the biopsy material was taken during EGD from the edge of the erosive area and the area of unchanged esophageal mucosa at a distance of two centimeters from the lower esophageal sphincter. Histological and immunohistochemical studies were performed with an assessment of histological signs of reflux esophagitis and proliferative activity of esophageal mucosa as well as evaluation of the tight junction proteins claudin-1, claudin-4 by immunohistochemistry.

Immunohistochemistry

The expression of the claudins' levels were assessed by a semi-quantitative system in the calculation of the expression index. The gradation of values represented assessment of the immune staining intensity: 1 point – weak intensity of the cell's membrane staining, 2 points – a moderate intensity, 3 points – pronounced intensity. As for the semiquantitative index, 1 point signified less than 10% of positively stained cells, 2 points - from 10% up to 50% of positively stained cells, 3 points - 51-80% of positively stained cells, 4 points - more than 80% of positively stained cells. Thus, in the case of expression, the index ranged from 1 to 12 points. Ki-67 expression was assessed as a percentage. To conduct an immunohistochemical reaction, paraffin sections were applied to adhesive glass slides with a positively charged surface. Temperature unmasking of antigens was carried out by heating in a water bath for 40 minutes in a solution of sodium citrate at pH=6.0. The antibodies claudin-1 – polyclonal rabbit antibodies, RTU (Cell Marque, USA), claudin-4 – monoclonal (clone 3E2C1) mouse antibodies (Biocare Medical, USA) were used with working dilution 1:100, proliferation marker Ki-67 – monoclonal (clone SP6) mouse antibodies (Cell Marque, USA) with working dilution 1:200. A biotin-free HiDef Detection HRP Polymer System (Cell Marque, USA) was used as an imaging system, followed by reaction results detection using a chromogenic DAB Substrate Kit (Cell Marque, USA).

Outcomes

The primary end point was the proportion (%) of patients in the group taking MPA and PPI and in the group taking PPI, who had healing of erosions after 4 weeks of treatment as determined by endoscopy.

The secondary endpoints of the study were the proportion (%) of patients in MPA and PPI group and in the PPI group who had an improvement in the endoscopic picture before and after treatment within 4 weeks by at least 1 level according to the Los Angeles classification, comparison scores on the proposed questionnaire for assessing the frequency and severity of GERD symptoms before and after 4 weeks of treatment in the group taking mucosal protection and PPI, and the group taking PPI, comparison of scores on the scale for assessing the expression of tight junction proteins (claudin-1 and claudin-4) and cellular proliferation marker (Ki-67) before and after 4 weeks of treatment in both groups. All endpoints were written in the study protocol.

Statistical Analysis

The sample size was determined by the Lehr formula. Mean sample values of quantitative traits were given in the text as median [lower quartile [Q1]; upper quartile [Q3]]. Categorical variables are expressed as percentages. To assess the normality of data distribution, the Kolmogorov-Smirnov and Shapiro-Wilk tests were used. Since the distribution in the samples did not meet the requirements of parametric analysis, nonparametric methods were used for statistical data processing. For unrelated samples, the Mann-Whitney U test was used (using Z-statistics for volumes n>20), and for linked samples, the Wilcoxon matched pairs test (using Z-statistics for volumes n>25). The frequencies were analyzed using the $\chi 2$ test. In all cases, the significance level α was set to a probability of less than 0.05. Statistical processing of the material was performed using the certified software package SPSS version 26.0 (IBM Inc., Chicago, Illinois, USA).

RESULTS

Participant Characteristics

62 patients with erosive esophagitis were examined, 60 of them were randomized; 30 participants in the MPA and PPI group and 30 participants in the PPI group. During the study, none of the participants dropped out due to the communication loss.

Table I presents the baseline demographic and clinical characteristics of the study groups.

When analyzing the occurrence and severity of GERD symptoms on the Likert scale in the study groups, no statistical differences were initially detected (p>0.05 for all symptoms).

When assessing the severity of the erosive esophagitis histological signs, the expression of Ki-67 and tight junction proteins, there were no statistically significant differences (p>0.05) between both groups on the region on of the edge of the erosive area and in the area of the unchanged mucosa.

Table I. Study participants

Indices	MPA + PPI group (n=30)	PPI group (n=30)	p
Age (years)	49 (38; 57)	39 (33; 51)	0.07
Gender (women) n, (%)	10 (33.33)	12 (40)	0.28
Disease precursors			
Onset age, years	44 (33.4; 52)	32,7 (24; 48)	0.51
Duration, years	5.5 (3; 7)	4 (2; 7)	0.54
Data on other risk factors			
Smoking (%)	8 (26.67)	6 (20)	0.08
BMI (kg/m^2)	30.98 (25.91; 34.89)	31.91 (27.18; 35.16)	0.38
BMI more than 25 kg/m^2, n, (%)	24 (80)	26 (86.67)	0.56
Hiatus hernia n, (%)	14 (46.67)	14 (46.67)	-

MPA: mucosal protective agent; PPI: proton pump inhibitor; BMI: body mass index.

During the endoscopic examination at the stage of inclusion in the group taking MPA and PPI, the degree C of erosive reflux esophagitis according to the Los Angeles classification was established in 22 (73.33%) participants, the degree D of erosive reflux esophagitis in 8 (26.67%) participants. In the PPI group, LA grade C erosive reflux esophagitis was found in 21 (70%) participants, and grade D erosive reflux esophagitis in 9 participants (30%). Group differences in stages of erosive reflux esophagitis according to the Los Angeles classification were not statistically significant (p>0.05).

Primary Endpoint

After 4 weeks from the start of therapy, a second endoscopic examination was performed using the "blinding" method (the endoscopist was not informed about the patient's therapy). Erosion healing was observed in three patients in MPA and PPI group (10%). Endoscopic remission in the PPI group was not achieved in any case. Improvement of endoscopy after treatment by at least 1 level according to the Los Angeles classification was noted in all 30 patients (100%) in the MPA and PPI group and in 24 patients (80%) in the PPI group (p=0.01).

Secondary endpoints

After 4 weeks of treatment, the incidence of heartburn (p=0.01), retrosternal pain (p=0.02), odynophagia (p<0.01) and dysphagia (p=0.02) was statistically significantly lower in the group taking PPIs and MPA than in the PPI-only standard dose group. The incidence of regurgitation (p=0.35), eructation (p=0.08) had no statistically significant differences. The incidence of GERD symptoms in the groups before and after treatment are presented in Table II.

After 4 weeks of treatment, in MPA and PPI group, the severity of retrosternal pain (p=0.04) and eructation (p=0.02) was statistically significantly lower than in the PPI group. The severity of heartburn (p=0.29) and regurgitation (p=0.69) after treatment was also slightly lower in MPA and PPI group, but no statistical differences were found with the PPI group. Due to the insufficient number of patients with dysphagia symptoms (2 participants in MPA and PPI group and 4 participants in the PPI group) and odynophagia (4 participants in MP and PPI group and 10 participants in the PPI group) after treatment, statistical differences were not assessed. The severity of GERD symptoms in the groups before and after treatment are presented in Table III.

Histological examination of biopsy material from the mucosa of the esophagus after combined therapy with PPI and MPA revealed a significant decrease in the severity of basal layer hyperplasia in the area of the erosive defect (p<0.01). However, outside the area of the erosive defect, the decrease in the severity of basal layer hyperplasia after treatment was not statistically significant (p=0.06). The decrease in the severity of ectasia of venules of connective tissue papillae, hydropic dystrophy, intercellular distances in the mucosa of the esophagus after treatment at both points of biopsy was statistically significant (p<0.05).

In the group taking PPIs, there was a decrease in the hyperplasia severity of the basal layer in the esophageal mucosa, in the area of the erosive defect (p=0.04). Outside the zone of the erosive defect, no significant decrease in the severity of hyperplasia of the basal layer was found (p=0.57). A statistically significant decrease in the severity of venular ectasia papillae was not detected at any of the points of taking biopsy material in the PPI group (p>0.05). The decrease in the

Table II. Incidence of GERD symptoms in the groups before and after treatment

Symptom	Symptom incidence, n (%)			
	MPA + PPI before treatment (n = 30)	MPA + PPI after treatment (n = 30)	PPI before treatment (n = 30)	PPI after treatment (n = 30)
Heartburn	28 (93.33)	17 (56.67)	28 (93.33)	22 (73.33)
Retrosternal pain	20 (66.67)	12 (40)	20 (66.67)	16 (53.33)
Regurgitation	23 (76.67)	15 (50)	26 (86.67)	15 (50)
Eructation	26 (86.67)	20 (66.67)	24 (80)	19 (63.33)
Odynophagia	18 (60)	4 (13.33)	16 (53.33)	10 (33.33)
Dysphagia	11 (53.33)	2 (6.67)	11 (36.67)	4 (13.33)

For abbreviations see Table I.

Table III. The severity of GERD symptoms in the groups before and after treatment

Symptom	Expression, points			
	MPA + PPI before treatment (n = 30)	MPA + PPI after treatment (n = 30)	PPI before treatment (n = 30)	PPI after treatment (n = 30)
Heartburn	4 (3; 4)	2 (2; 2)	3,5 (3; 4)	2 (2; 3)
Retrosternal pain	3 (2,5; 4)	2 (2; 2)	3 (3; 4)	2 (2; 3)
Regurgitation	4 (3; 4)	2 (2; 3)	4 (3; 4)	2 (2; 3)
Eructation	3 (2; 3)	2 (2; 2)	3 (2; 3)	2 (2; 3)
Odynophagia	3 (2; 4)	2 (2; 2)	3 (2; 4)	2 (2; 2)
Dysphagia	3 (2; 4)	2 (2; 2)	3 (2; 4)	2 (2; 2,5)

For abbreviations see Table I.

severity of hydropic dystrophy in the zone of the erosive defect also turned out to be insignificant (p=0.08). However, outside the zone of the erosive defect, the decrease in the severity of hydropic dystrophy was statistically significant (p=0.01). The decrease in the severity of intercellular distances in the mucosa of the esophagus in the area of the erosive defect was significant (p=0.03), but no statistically significant improvements were found outside the area of the erosive defect (p=0.14). The severity of histological changes in the groups before and after treatment are presented in Table IV.

Comparative evaluation of the histological examination results of the biopsy material after 4 weeks of therapy in the area of the erosive defect revealed a large reduction in basal hyperplasia (p=0.03), venular ectasia (p=0.04), and expansion of intercellular distances (p=0.03) in MPA and PPI group, comparing to the PPI group. Outside the zone of erosive mucosal defect, the difference was not statistically significant (p>0.05). The severity of dystrophic changes in both groups after 4 weeks of therapy did not differ significantly (p>0.05). The esophageal histopathological changes in patients of both groups in the area of the erosive defect 4 weeks after the start of therapy is shown in Fig. 2.

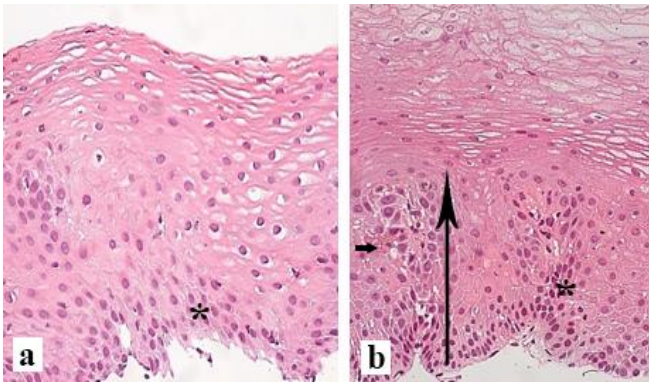

Fig. 2. Esophageal mucosa in the area of the erosive defect 4 weeks after the start of the therapy. a –biopsy of a patient from MP and PPI group. Weak hyperplasia of the cells in the basal layer (asterisk). In the basal and spiny layer, the expansion of intercellular contacts is irregular and weakly expressed. Stained with hematoxylin and eosin. x200. b - biopsy of a patient from the PPI-only group. The hyperplasia of the basal layer (asterisk), venular ectasia (arrow), the height of the stromal papillae is less than 50% of the height of the epithelial layer (long arrow). Hematoxylin and eosin. x200.

A significant decrease in the expression of the Ki-67 proliferation marker was found in the area of the erosive defect (before treatment vs after treatment, 14 (7; 18) vs 9 (6; 12), p=0.01) and outside the area of the erosive defect of the esophageal mucosa (before treatment vs after treatment, 11 (8; 17) vs 10 (8; 13), p=0.02) in the group treated with MPA and PPIs. In the PPI group, after 4 weeks of treatment, there was no statistically significant decrease in the level of proliferative activity in the area of the erosive defect (before treatment vs after treatment, 15 (8; 17) vs 12 (8; 15), p=0.25) and outside the zone of an erosive defect of the esophagus mucosa (before treatment vs after treatment, 13 (7; 16) vs 10 (7; 14), p=0.36). The expression level of the Ki-67 marker in the area of the erosive defect was statistically significantly lower in the group treated with MPA and PPI than in the group treated with PPI (p=0.04). Immunohistochemical features is illustrated in Fig. 3. Outside the area of the erosive defect, differences in the expression of the Ki-67 proliferation marker after treatment were not statistically significant (p=0.75).

When assessing the state of tight junction proteins of the esophageal mucosa in the group treated with MPA and PPIs, a significant increase in the expression of claudin-1 in the area of the erosive defect was found [before treatment vs after treatment, 4 (2; 7) vs 8 (6; 9), p=0.01] and outside the area of the erosive defect of the esophageal mucosa [before treatment vs after treatment, 5 (4; 6) vs 9 (5; 10), p=0.01]. An increase in the expression of claudin-1 proliferative activity in the area of the erosive defect in the PPI group also found after treatment [4 (3; 6) vs 5 (4; 8), p=0.04)] and beyond zone of an erosive defect of the mucosa of the esophagus [5 (4; 8) vs. 6 (4; 8), p=0.01). The severity of claudin-1 expression in the area of the erosive defect of the esophageal mucosa 4 weeks after the start of therapy is shown in Fig. 4. The expression level of claudin-1 in the area of the erosive defect was statistically significantly higher in the group treated with MPA and PPI than in the group treated with PPI (p=0.03). Outside the area of the erosive defect, the differences in the expression of claudin-1 were not statistically significant (p=0.37).

In the group treated with MPA and PPIs, a significant increase in claudin-4 expression was found in the area of the erosive defect after treatment [2 (1; 4) vs 6 (5; 8), p<0.01] and beyond zone of erosive defect [4 (3; 5) versus 6 (5; 7), p<0.01].

Table IV. The severity of histological changes in the groups before and after treatment

Trait	Biopsy collection point	Trait expression, points			
		MP + PPI before treatment (n = 30)	MP + PPI after treatment (n = 30)	PPI before treatment (n = 30)	PPI after treatment (n = 30)
Basal layer hyperplasia	point 1	2 (1; 2)	1 (0; 1)	2 (1; 2)	1 (1; 1)
	point 2	1 (1; 1)	1 (0; 1)	1 (1; 2)	1 (1; 1)
Ectasia of venules of connective tissue papillae	point 1	1 (1; 2)	1 (0; 1)	1 (1; 2)	1 (1; 1)
	point 2	1 (1; 2)	1 (1; 1)	1 (1; 2)	1 (1; 1)
Hydropic dystrophy of epitheliocytes	point 1	1 (1; 2)	1 (1; 1)	1 (1; 2)	1 (1; 1)
	point 2	2 (2; 2)	1 (1; 2)	2 (1; 2)	1 (1; 1)
Extension of intercellular distances	point 1	2 (1; 2)	1 (1; 1)	2 (1; 2)	1 (1; 2)
	point 2	1 (1; 2)	1 (1; 1)	1 (1; 2)	1 (1; 1)

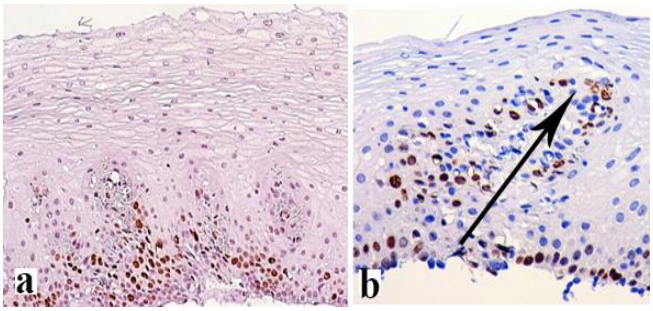

Fig. 3. Proliferative marker Ki-67 expression in the area of the erosive defect of the esophageal mucosa 4 weeks after the start of the therapy. a - biopsy of a patient from MPA and PPI group. Decreased index of proliferative activity. x200. b- biopsy of a patient from the PPI-only group. Preservation of high stromal papillae (long arrow). The proliferative activity index is reduced in comparison with before treatment, but higher than in MPA and PPI group. x300.

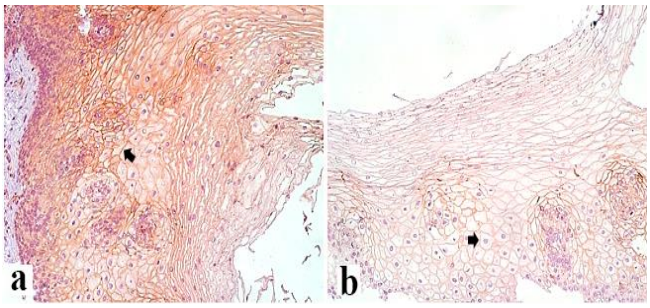

Fig. 4. Claudin-1 expression in the area of the erosive defect of the esophageal mucosa 4 weeks after the start of the therapy. a - biopsy of a patient from MPA and PPI group. A pronounced immunohistochemical reaction with an increase in the expression of the claudin-1 protein (brown membrane mark, arrow). x300. b - biopsy of a patient from the PPI-only group. Moderate intensity of immunostaining (arrow). x300.

In the PPI group, an increase in the expression of claudin-4 activity in the area of the erosive defect was also found [3 (2; 4) vs 4 (4; 6), p=0.01] and beyond the zone of an erosive defect of the esophagus mucosa [4 (3; 4) vs 5 (4; 6), p<0.01]. The expression level of claudin-4 in the zone of the erosive defect was statistically significantly higher in the group treated with MPA and PPI than in the group treated with PPI (p=0.02). Outside the area of the erosive defect, the expression of claudin-4 was higher in the group treated with MPA and PPIs in the zone of the erosive defect and outside (p=0.02). Expression level of the Ki-67 marker, claudin-1 and claudin-4 in the area of the erosive defect and outside the erosive area before and after treatment in both groups are presented in Fig. 5.

DISCUSSION

It is known that tight junction proteins, in particular, claudin-1 and claudin-4, are involved in maintaining the integrity of the mucosal barrier of the esophageal mucosa, whose disturbance leads to an easier penetration of aggressive components of the refluxate into the subepithelial layer and the development of inflammation. The data available in modern literature suggest that the nature of damage to the esophageal mucosa depends not so much on the damaging properties of the refluxate itself, but on the state of the mucosal barrier of the esophageal mucosa [5, 10, 11].

Damage to the esophageal mucosa under the action of refluxate also leads to an increase in the proliferative potential

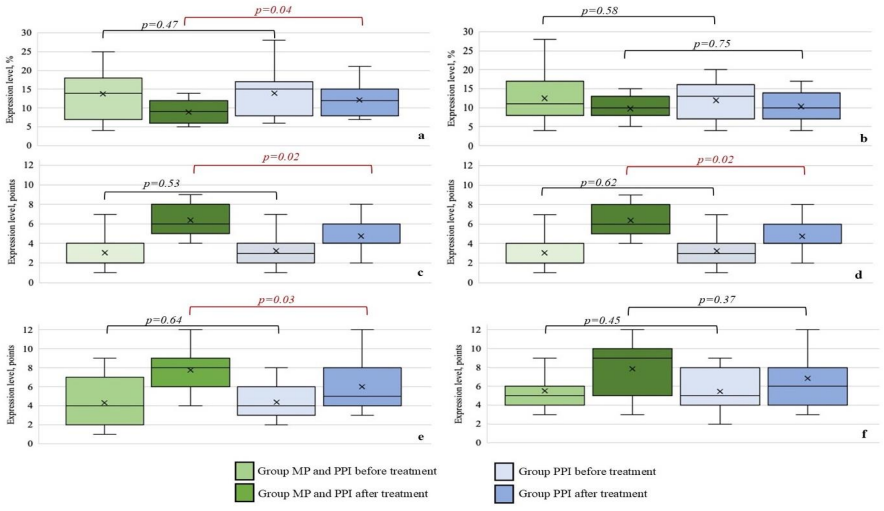

Fig. 5. Expression level of the Ki-67 marker, claudin-1 and claudin-4 in the area of the erosive defect and outside the erosive area before and after treatment in both groups: a - expression level of the Ki-67 marker in the area of the erosive defect's edge before and after treatment in both groups; b - expression level of the Ki-67 marker outside the erosive area before and after 4 weeks of treatment in both groups; c - claudin-4 expression level in the erosive area before and after 4 weeks of treatment in both groups; d - expression level of claudin-4 outside the erosive area before and after 4 weeks of treatment in both groups; e - claudin-1 expression level in the erosive area before and after 4 weeks of treatment in both group; f - claudin-1 expression level outside the erosive area before and after 4 weeks of treatment in the MPA and PPI group and the PPI group.

and hyperplasia of the basal layer. A high proliferative potential is important in assessing the prognosis for the risk of intestinal metaplasia and epithelial dysplasia. Expression of the Ki-67 protein is a classic marker of cell proliferation and has been fairly well studied in GERD. A number of studies have shown that Ki-67 expression increases in parallel with an increase in the severity of morphological changes in the esophageal mucosa along the progression path from reflux esophagitis to Barrett's esophagus and esophageal adenocarcinoma [12].

Thus, the decrease in the expression of claudin-1 and claudin-4 detected in the course of our study against the background of increased expression of Ki-67, both on the edge of the erosive defect and in the area of the unchanged mucosa, indicates a reduced resistance of the esophageal mucosa to the effects of the reflux aggressive molecules, which increases the risk of an erosive form of GERD development.

After 4 weeks from the start of therapy in both groups, a statistically significant decrease in the frequency and severity of the characteristic clinical manifestations of GERD was found, like heartburn, retrosternal pain, regurgitation, eructation, odynophagia and dysphagia.

A decrease in the expression level of the proliferation marker Ki-67 after treatment was also established both in the area of the erosive edge and in the area of the unchanged mucosa at a distance of two centimeters from the lower esophageal sphincter in all patients, while in the intergroup comparison these differences were not statistically significant. At the same time, after treatment, in the group taking MPA and PPI, a significant increase in the expression level of claudin-1 and claudin-4 was recorded both in the region of the erosive area edge and in the area of the unchanged mucosa. The revealed changes in the form of the expression decrease in the cell proliferation marker Ki-67 against the background of a significant increase in the expression of the studied proteins of tight junctions (claudin-1, claudin-4) indicate a more effective restoration of the barrier function in the esophageal mucosa and prevent the formation of complications in a patient with an erosive form of GERD when prescribing a combination therapy of PPI and MPA.

Limitations

The presence of inclusion and exclusion criteria did not permit the evaluation of the efficacy and safety of combination therapy with PPIs and MPA in asymptomatic patients, patients with non-erosive GERD, persons with Barrett's syndrome, concomitant gastric and duodenal ulcers. The absence of a group with healthy participants did not allow us to assess the severity of morphological and immunohistochemical changes in individuals with erosive esophagitis before and after therapy in comparison with healthy ones.

CONCLUSION

Individualized approach based on the use of combination therapy with PPIs and MPA makes it possible to increase the effectiveness of treatment in achieving clinical, endoscopic and, more importantly, histological remission in a patient with erosive GERD.

The presented study also opens up prospects for a further study of the esophageal mucosa state. We did not set the task of evaluating the outcomes and the progression rate of the disease. It seems interesting to what extent the remaining high proliferative potential of the esophageal mucosa in patients with erosive GERD, which we identified in this study, will serve as a starting point for the formation of Barrett's esophagus and associated esophageal adenocarcinoma. In addition, it is possible to conduct longer randomized studies in order to clarify for how long the achieved results in the treatment of erosive esophagitis with MP and PPIs could be preserved.

Conflicts of interest: None to declare.

Author's contributions: D.S.B., M.A.L. and S.I.M. conceived and designed the study. M.A.L, S.I.M. O.V.G amd I.V.L. were responsible for data analysis and interpretation, and manuscript drafting. D.S.B., M.A.L., S.I.M and O.V.G. critically revised the article for important intellectual content. All the authors reviewed and approved the final version to be published.

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