A randomized, double-blind, placebo-controlled pilot study to evaluate the efficacy and tolerability of a novel oral bioadhesive formulation for the treatment of nonerosive reflux disease-related symptoms

A randomized, double-blind, placebo-controlled pilot study to evaluate the efficacy and tolerability of a novel oral bioadhesive formulation for the treatment of non-erosive reflux disease-related symptoms

Short title: NERD and a novel oral bioadhesive formulation

Davide Giuseppe Ribaldone[a], Pendlimari Rajesh[b], Divya Chandradhara[c], Marco Astegiano[d] and Rinaldo Pellicano[d*]

The authors (DGR, PR) contributed equally to this work

[a]Department of Medical Sciences, University of Turin, Turin, Italy

[b]Rajalakshmi Hospital, Vidyaranyapura post, Bangalore, India

[c]Bioagile Therapeutics Pvt. Ltd., Bangalore, India

[d]Unit of Gastroenterology Molinette and San Giovanni Antica Sede Hospitals, Città della Salute e della Scienza, Turin, Italy

[*]Corresponding author: Rinaldo Pellicano, MD, Unit of Gastroenterology, Molinette-SGAS Hospital, Via Cavour 31, 10126 Turin, Italy. E-mail: rinaldo_pellican@hotmail.com

Conflicts of Interest and Source of Funding: The authors have no conflicts of interest. The study was supported by Giellepi S.p.A. (Lissone, MB, Italy).

Abstract

Objective. The use of antisecretory drugs can provide symptomatic relief in 70–80% of patients suffering from gastro-oesophageal reflux disease (GORD), although this benefit is reduced by 20–30% in the case of non-erosive reflux disease (NERD). The current study evaluates the efficacy and safety of a patented oral formulation (liquid sachets containing hyaluronic acid, a mixture of amino acids including proline, hydroxyl-proline and glutamine, and rice extract dispersed in a bioadhesive polymer matrix) for relieving the symptoms of NERD.

Methods. A single-centre, randomized, double-blind, parallel group, placebo-controlled clinical study was performed. Patients who experienced at least 3 episodes of moderate-severity heartburn during the 7-day run-in period were included and treated with 3 liquid sachets per day for 14 days. The primary objective was to evaluate the proportion of patients with at least a 3-point reduction in the total symptom score (TSS).

Results. Overall, 20 patients were randomized to receive the investigational product and 20 to receive the placebo. At the end of treatment, a 3-point reduction in the TSS was achieved by 95% of patients treated with the investigational product and by 20% of patients treated with placebo ($P < 0.0001$). No adverse events were reported.

Conclusions. The investigational product showed a statistically significant superiority to the placebo in relieving common symptoms in patients with NERD. Future studies will be aimed at clarifying the hypothesis that this symptomatic benefit is related to the strengthening of the oesophageal barrier against the damage induced by gastric contents.

Key words: amino acids; GORD; hydroxyproline; Oryza sativa; PPI; proline.

Introduction

Gastro-oesophageal reflux disease (GORD), defined as troublesome symptoms or mucosal damage resulting from the reflux of gastric content into the oesophagus, is one of the most prevalent gastrointestinal tract disorders. This disease has an estimated prevalence of **14.8% that ranges from 7.4% in South-East Asia to 21.3% in Southern European studies and 22.1% in South Asian studies** [1]. The most specific symptoms of GORD are heartburn and regurgitation [2]. Other oesophageal and extra-oesophageal symptoms include dysphagia, globus sensation, odynophagia, chronic cough, asthma, hoarseness, wheezing and nausea [3,4]. These troublesome symptoms could impair the patient's quality of life, and injury or complications can result from the retrograde flow of gastric contents into the oesophagus, oropharynx and/or respiratory tract [5].

There are two main forms of GORD called erosive reflux disease (ERD) and non-erosive reflux disease (NERD); the latter represents up to 70% of patients with typical reflux symptoms and is characterized by the absence of visible mucosal lesions during upper gastrointestinal endoscopy [6]. In the past decades, several experts have suggested that the diagnosis of NERD should be supported by evidence that symptoms were caused by acid reflux; this can be evaluated on the basis of excess acid in the oesophagus, a positive correlation between reflux symptoms and acid reflux episodes based on pH testing, or a good response to acid suppression therapy with proton pump inhibitor (PPI) drugs [7]. More recently, the use of 24-hour oesophageal impedance-pH monitoring has shown that weak acidic reflux is also able to induce typical and atypical GORD symptoms, which, however, do not respond to PPIs [8]. Furthermore, non-acidic reflux is also associated with

histopathological alterations consisting of the dilation of intercellular spaces between adjacent cells of the oesophageal epithelium; these have been documented by electron and light microscopy in the majority of NERD patients [9]. Such evidence supports the hypothesis that this clinical condition is not *sine materia* as it is associated with a disruption of the physiological oesophageal mucosal barrier. This latter normally consists of an impermeable multilayer of the squamous epithelium that prevents both the intercellular transit of noxious refluxed contents and their contact with nociceptive afferents, thus avoiding their excitation and consequent heartburn. The molecular basis of acid-induced symptoms is controversial since these could be attributed to the activation of the transient receptor potential vanilloid subfamily member 1 (TRPV1), which is present in nociceptive afferents and possibly in epithelial cells, although this remains unconfirmed [10]. In fact, while some studies have reported an upregulation of the *TRPV1* gene in the mucosa of NERD patients, others were unable to confirm these findings. Based on the former theory, activation of this receptor would result in the release of neuropeptide transmitters and inflammatory mediators with the induction of both acid hypersensitivity and low-grade inflammation [10]. Furthermore, it has been shown in an animal model that inflammation (measured by pro-inflammatory cytokine levels), in combination with impaired permeability, is a co-factor associated with NERD pathogenesis [11].

Nowadays, relief of symptoms can be achieved in 70–80% of patients with GORD through adequate medical therapy, although this benefit is reduced by 20–30% in those with NERD because of a different pathophysiological pathway [6]. In recent years, particular attention has been paid to the role of both weak mucosal defences and

morphology changes during induction of symptoms, particularly in patients with NERD. Thus, strengthening the oesophageal mucosal resistance could be a potential therapeutic target in the treatment of this condition.

The aim of the current study was to assess the efficacy, safety and tolerability of a liquid formulation for oral intake for the management of NERD symptoms. This formulation contains hyaluronic acid, a mixture of amino acids including proline, hydroxyl-proline and glutamine, and rice extract dispersed in a bioadhesive polymer matrix; these components act in synergy, binding to the oesophageal mucosa and exerting a calming and soothing effect on the irritated tissue. Additionally, hyaluronic acid and amino acids should preserve the integrity of the tissue.

Methods

This single centre, randomized, double-blind, parallel group, placebo-controlled clinical study was performed at Rajalakshmi Hospital in Bangalore (India). Forty adult subjects, **referring for persistent symptoms typical for GORD,** were enrolled after signing the informed consent form; inclusion and exclusion criteria are shown in tables 1 and 2.

The product that is the subject of this study is a patented medical device (class IIa) developed by Giellepi S.p.A. Health Science (Lissone, MB, Italy). The product contains hyaluronic acid, amino acids (i.e. proline, hydroxyl-proline, glutamine) and rice extract (VGF®) dispersed in a bioadhesive polymer matrix that binds to the oesophageal epithelial cells and prolongs the contact of the components with the target tissue. The investigational product is packaged in ready-to-take sachets containing a single dose of liquid solution (10

ml) for oral administration. **Placebo contains xanthan gum and all the excipients of the investigational product which are sucralose, flavor and preservative, in purified water.** Placebo sachets were of identical appearance and matched for taste and viscosity.

After the initial screening (visit 1), subjects were randomly assigned to each arm equally using a blocked randomization method. **The randomization code list was generated using a computer-generated randomization list with the SAS 9.4 software.** Three sachets per day (shortly after the main meals and before going to sleep) of investigational product or placebo were administered for 14 days to each patient.

The primary outcome was to evaluate the efficacy of treatments in terms of the proportion of patients with a significant remission of NERD symptoms (heartburn and regurgitation), defined as a total symptom score (TSS) reduction of at least 3 points. This was calculated by collecting and computing the intensity/severity of each patient's symptoms based on the Reflux Disease Questionnaire (RDQ) and comparing the baseline values with those reported at the end of the study. TSS was calculated by adding all the scores from the intensity/severity of GORD symptoms (heartburn and regurgitation) using the RDQ questionnaires obtained at Day 0 (baseline, visit 2), Day 7 (visit 3) and Day 14 (visit 4-end of treatment). Symptoms related to NERD (heartburn, acid regurgitation, retrosternal pain and acid taste in the mouth) were rated by patients on a 5-point Likert scale as follows: 0 = no symptoms, 1 = slight symptoms, 2 = moderate symptoms, 3 = severe symptoms, and 4 = very severe symptoms. Pain was assessed through the use of a Visual Analogue Scale (VAS, a 0-10 point scale).

Secondary outcomes were as follows:

• safety and tolerability of the investigational product;

• number of patients with a 50% reduction of symptoms at the end of treatment;

• number of days required to achieve the first 24 hours without heartburn;

• number of days required to achieve the first daytime or night-time heartburn-free interval;

• reduction of the Heartburn Severity Index (sum of all individual episodes of daytime and night-time heartburn per severity score per episode);

• improvement of Health-Related Quality of Life (HRQL) according to the Short Form-36 (SF-36) questionnaire.

Initially, eligible patients were entered into a 1-week run-in period during which they maintained a daily diary of NERD symptoms. This included recording the name and number of administered medications and doses, daily severity of symptoms (Questionnaires using a Likert scale and VAS scale), time elapsed to onset of action, and any consumption of rescue medication (antacids). Other details taken and recorded in the case report form (CRF) were date of birth, gender, race, weight, height, and smoking and drinking habits. An upper gastrointestinal endoscopy (if not done in the last 3 months prior to screening) was performed during the screening (Days -7 to 0) to rule out the presence of oesophageal erosions. Each patient compiled the symptom diary daily during the study period, recording the presence (including severity) or absence of each symptom during the day and the night. Patients were told to refrain from using rescue medications (antacids) unless symptoms were severe enough to be intolerable, and the number of tablets consumed daily were recorded. Participants and physicians were blinded regarding whether patients would be taking active product or placebo.

Any adverse event (AE) that occurred during the study or the follow-up period was recorded in the subject's CRF. The safety population included all randomized subjects who had taken the study product at least once.

Finally, patients' compliance was calculated based on the test product used, which was obtained by counting the returned medications at the end of the study. A treatment compliance of ≥ 80% was considered acceptable for statistical evaluation.

Ethical and statistical information

The study was conducted according to the Good Clinical Practice guidelines, as issued by the International Conference on Harmonization (ICH/135/95, July 2002) guidelines, the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and ISO 14155 regarding the clinical investigation of medical devices for human subjects – Good clinical practice.

The study protocol and informed consent were approved prior to study initiation by the Rajalakshmi Hospital Institutional Ethics Committee, Bangalore, India.

The trial was registered in the Clinical Trials Registry of India with the following number: CTRI/2018/06/014504.

Statistical Analysis

The primary outcome was calculated by collecting and computing the intensity/severity of each patient's symptoms (on the basis of the RDQ questionnaire) at the final visit and comparing it with the baseline values obtained at the end of the run-in period.

Summary statistics and ANCOVA/ANOVA analysis were applied to the related data; intragroup analysis was performed in order to evaluate the remission of symptoms at the end of treatment compared to baseline (ANOVA analysis). Continuous variables were reported as the mean or median depending on data distribution. The normality of the data was evaluated by the D'Agostino-Pearson test. The comparison of continuous variables between independent groups was performed using the independent-samples t-test or the Mann-Whitney test. The comparison of paired measurements was carried out using the student's t-test for paired measurements or the Wilcoxon test, depending on the distribution of data. The Fisher's exact test was performed for dichotomous qualitative variables. For all analyses, $P < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS software version 22.0 (IBM SPSS Statistics for Windows, Chicago, IL).

To determine the sample size, the type I error and power were considered 0.05 and 80% respectively. The sample size was calculated based on the reduction of the NERD TSS by 3 points at the final visit and assuming a rate of 20% for patients with a TSS reduction of at least 3 points in the placebo group and 65% in the investigational product group. A power level of 80% with a significance value ≤ 0.05 (two-sided Fisher's exact test) required a sample size of 18 patients for each group. Considering non-evaluable patients to be 12%, the sample was raised to 20 patients for each group.

Results

A total of 55 subjects were screened, of whom 40 were enrolled and randomized into 2 treatment arms: 20 patients were treated with investigational product and 20 with placebo (Figure 1). Clinical and demographic features of patients included in the two groups were comparable (Table 3). All subjects completed the study without withdrawals/drop-outs and a treatment compliance ≥ 80% (range 93% - 100%) was reported by all participants.

Primary outcome

The proportion of patients with a TSS reduction of at least 3 points among those treated with the investigational product was 95% (19 out of 20 patients). The proportion of patients with a TSS reduction of at least 3 points among those in the placebo group was 20% (4 out of 20 patients) ($P < 0.0001$) (Figure 2).

The mean TSS (mean ± standard deviation, SD) for patients treated with the investigational product at Day 0 (Baseline), Day 7 and Day 14 was 14.4 ± 2.2, 9.2 ± 4.9 ($P < 0.001$, compared to baseline) and 4.6 ± 2.9 ($P < 0.0001$, compared to baseline), respectively. Thus, TSS decreased by 68.3% in subjects treated with the investigational product. The mean TSS for patients in the placebo group at Day 0 (Baseline), Day 7 and Day 14 was 14.8 ± 3.1 ($P = 0.6$ compared to the investigational product), 15.9 ± 3.1 ($P = 0.000007$, compared to investigational product) and 15.2 ± 2.8 ($P < 0.0001$, compared to the investigational product), respectively. Thus, TSS increased by 2.4% during treatment with placebo (Figure 3).

Secondary outcomes

As regards safety and tolerability of both the investigational product and the placebo, no adverse events were reported during the entire study.

In terms of efficacy, 18 out of 20 subjects (90%) in the investigational product group had a 50% TSS reduction *versus* none (0%) in the placebo group ($P < 0.0001$). Regarding the number of days required to achieve the first 24 hours without heartburn, 50% of patients (10/20) showed a 24-hour heartburn-free interval after 9 days of investigational product intake while no improvement was reported in the placebo group ($P = 0.0003$). Regarding the time to achieve the first daytime or night-time heartburn-free interval, 50% of subjects (10/20) showed a 12-hour (first daytime or night-time) heartburn-free interval after 9 days of investigational product intake while no improvement was reported in the placebo group. Heartburn severity index was found to be statistically lower among patients treated with investigational product compared to those in the placebo group at Day 7 (4, SD = 2, *versus* 6.3, SD = 0.9, $P < 0.0001$) and at Day 14 (2.1, SD = 1.1, *versus* 6, SD = 1.4, $P < 0.0001$) (Figure 4).

The quality of life assessment was carried out using a SF-36 questionnaire at the baseline (Day 0) and at the final visit (Day 14). Regarding the improvement in physical activity, the median score for patients in the investigational product group at baseline and at Day 14 was 65 (95% confidence interval, CI = 50.8 – 84.2) and 85 (95% CI = 55 – 95), respectively ($P = 0.006$); the median score in the placebo group at baseline and at Day 14 was 50 (95% CI = 30 – 54.2) and 50 (95% CI = 30 – 50), respectively ($P = 0.63$). Assessing the physical role limitations, the median score for patients undergoing investigational product treatment at baseline and Day 14 was 50 (95% CI = 0 – 100) and 100 (95% CI = 100 – 100),

respectively (*P* = 0.001), *versus* 0 (95% CI = 0 – 95.8) and 0 (95% CI = 0 – 100) in the placebo group. **Considering the number of patients who reported a reduction of 50% in physical pain,** the median score for patients undergoing investigational product treatment at baseline and Day 14 was 30 (95% CI = 30 – 41) and 61 (95% CI = 61 – 74), respectively (*P* = 0.001), *versus* 30 (95% CI = 30 – 30) and 30 (95% CI = 30 – 30) in the placebo group. The evaluation of general health parameters showed a median score for patients undergoing investigational product treatment at baseline and Day 14 of 36 (95% CI = 30 – 41.2) and 57 (95% CI = 52 – 61), respectively (*P* = 0.0008), *versus* 30 (95% CI = 30 – 35) and 35 (95% CI = 30 – 41.7)(*P* = 0.06) in the placebo group. Furthermore, the vitality assessment ameliorated significantly in the treatment group, with a median score increasing from 40 at baseline to 60 at Day 14 (*P* = 0.0004), while no change was reported in the placebo group (it remained 40). In the investigational product group, social activities improved from a median score of 50 (95% CI = 37 – 50) at baseline to 62 (95% CI = 50 – 72.8) at Day 14 (*P* = 0.0006), respectively. This score remained unchanged (50) in the placebo group (*P* = 0.84). Considering emotional role limitations, the median score for patients in the investigational product group at baseline and Day 14 was 33 (95% CI = 0 – 100) and 100 (95% CI = 100 – 100), respectively (*P* = 0.001). In the placebo group, the median score increased from 0 (95% CI = 0 – 94.2) at baseline to 33 (95% CI = 0 – 100) at Day 14, respectively (inestimable *P* due to small sample size). The mental health assessment showed that the median score in the investigational product group increased from 44 at baseline to 60 at Day 14 (*P* = 0.005). This median decreased from 48 to 40 in

the placebo group ($P = 0.03$). Table 4 reports the median scores of the quality of life parameters.

Thirty-six patients used rescue medication (antiacids) at least one day during the first week of the study (20 subjects in the placebo group and 16 in the active group). On the contrary, 22 patients used rescue medication (antiacids) at least one day during the second week of the study (20 subjects in the placebo group and 2 in the active group).

Discussion

The current medical management of GORD is based on the administration of acid secretion inhibitors such as PPIs. These drugs provide an 80–85% healing rate of oesophageal lesions, including ulcers, and also reduce the incidence of complications [12]. Pooled analyses have shown that symptom relief can also be achieved in 56–76% of cases, although this benefit is reduced in patients with NERD [13,14].

During an endoscopy, 60% of patients with typical GORD symptoms do not present evidence of mucosal damage (NERD); this underlines the importance of managing this condition.[6] A large American Gastroenterological Association survey found that despite PPI use, over 55% of subjects with GORD symptoms in the general population have reported continued impairment of their quality of life [15]. Recent studies have demonstrated that non-acidic reflux has a crucial role in contributing to histopathological alterations and triggering symptoms [16]. In this context, a synergistic action between acid and duodenogastric reflux has been suggested. The latter, with its bile contents, enhances

the damage caused by acid or induces injury itself [17]. Therefore, there is a need for new treatment options for GORD patients, especially when complete resolution of symptoms is considered an endpoint.

An ideal therapy for NERD patients, in addition to mitigating acid secretion, should provide a barrier to the residual aggressive components of the refluxate (i.e. weakly acidic and non-acidic gastric juice contents) while also stimulating mucosal repair. There are only a few products potentially combinable with PPI that are available today. These include irsogladine (a mucosal protective compound) [18], alginate-containing formulations [19] and hyaluronic acid-chondroitin sulphate based bioadhesive formulations [20,21], which have improved symptom control in NERD patients. A new oral bioadhesive formulation, registered in Europe as a class IIa medical device, that combines hyaluronic acid, a mix of amino acids (such as proline, hydroxyl-proline and glutamine) and rice extract, may constitute a modern approach to treating GORD cardinal symptoms. Hyaluronic acid, amino acids and rice extract are dispersed in a bioadhesive polymer matrix that enables the delivery of the functional ingredients to the oesophageal mucosa. This bioadhesive action is a fundamental attribute of the formulation that supports the product's long-lasting action. Hyaluronic acid is an anionic polysaccharide that is widely distributed throughout the extracellular matrix of connective, epithelial and neural tissues; it consists of a linear chain of D-glucuronic acid and N-acetyl-glucosamine fragments. Through its interaction with specific receptors, hyaluronic acid is involved in several key processes such as control of epithelial cell turnover, acceleration of re-epithelialization and mucosal hydration in ulcer healing [21]. Hyaluronic acid's hygroscopic nature enables it to form a scaffold that several

sulphur proteoglycans can bind to. Such structures can reach a large size and trap large quantities of water and ions, providing hydration and tissue distension [22]. In fact, hyaluronic acid-based hydrogels are used as scaffolds to facilitate tissue repair or regeneration at sites of injury and are degraded by tissue enzymes after repair is completed. For example, in the oral mucosa, this property either enables the control of tissue hydration during inflammation processes or contributes to halting tissue injury which results in ulcer formation. Hence, topical hyaluronic acid formulations are employed to treat recurrent aphthous ulceration of the oral mucosa and to achieve rapid symptom relief [20]. The other components of this product are novel in their mechanism of action as well as their assemblage. Amino acids promote the physiological repair of oesophageal mucosa damaged by contact with gastric contents. Their regenerative effect, aside from participating in the protein synthesis of wound tissue, could be realized by an anti-inflammatory prolonged action on the oesophageal layer. Rice extract exerts a calming and soothing effect on the irritated tissue.

The recently published Indian consensus on GORD in adults reported the most recent data on this issue. In this paper, it is highlighted that GORD occurs in almost 10% of both the rural and urban populations in India suggesting that the Indian prevalence of GORD is quite comparable to that in the Western countries and is higher than in many Asian countries [23]**.**

In this single centre, randomized, double-blind, parallel group, placebo-controlled clinical study, 20 patients were treated with investigational product and 20 with placebo. The results showed that 19 patients had a 3-point reduction in TSS (indicator of

intensity/severity of GORD symptoms) after treatment with the investigational product whereas 4 patients had a 3-point reduction in TSS in the placebo group. The total symptom score decreased by 68.3% among patients treated with the investigational product. Thus, the efficacy of the investigational product was significant compared with the placebo, which did not induce significant benefits. Furthermore, quality of life improved remarkably among patients treated with the investigational product and no adverse events were reported. Thus, in patients with NERD, the investigational product induced mucosal protection that led to both symptom relief and improvement of HRQL. The finding that the Heartburn Severity Index was statistically lower among patients treated with the investigational product could be explained by its mechanism of action, which enhances the oesophageal barrier with its bioadhesive formulation and protects the epithelium from gastric contents.

The composition of the investigational product could justify its fast action, witnessed by the achievement after 9 days of the first 24 hours without heartburn. The poor clinical evolution found among patients in the placebo group could be due to a statistical effect induced by the small sample size.

Several limitations of our study merit discussion. Given that a functional investigation (i.e. pH-impedance recording) was not performed, it is possible that the population studied included patients with functional heartburn and reflux hypersensitivity. In this context, the Rome IV consensus has subdivided the NERD phenotype into true NERD when acid exposure can be documented, and into reflux hypersensitivity and functional heartburn in the absence of evident acid exposure [24]. Although many aspects of hypersensitivity and

functional heartburn remain unclear, their pathogenesis is thought to be associated with peripheral or central sensitization. Due to the absence of pathological refluxate, psychological factors such as stress, followed by increasing oesophageal permeability, are believed to have a prominent role [25]. Additionally, although it was adequately powered to show a significant effect, this was a relatively small trial **and the TSS score used was not previously validated.** Finally, there was not a group of patients in PPI therapy. Nevertheless, the possibility of using the investigational product in combination with conventional PPI therapy is plausible from a biological point of view due to its mechanism of action (mechanical and non-pharmacological), which should not cause interaction with drugs. This combination therapy could be an appropriate strategy for patients with NERD who fail to respond to PPI treatment since the lack of response could be due to non-acid refluxates, which are better managed with a product that acts against both acid and non-acid components of the gastric juice. Given the unique features of the investigational product, which facilitate increased protection for the intercellular spaces and the nociceptive receptors, it is possible that a combination therapy with PPI agents could ameliorate symptoms by both reducing gastric contents and enhancing the oesophageal barrier. Finally, the investigational product could also be used in case of intermittent PPI therapy. This strategy could both reduce the risk of long-term side effects of PPI drugs, which have raised several concerns in recent years [12], as well as lower costs.

Future studies should evaluate the long-term effects of the investigational product, focusing on the patient's quality of life, on improving the efficacy of PPI, on the fewest days of work lost and on the lesser use of resources. Furthermore, since there is a variable

risk of progression from NERD to GORD [26], it would be interesting to evaluate whether a product intended to exert a long-acting symptomatic, anti-inflammatory and regenerative effect could aid in preventing the evolution towards erosive disease.

In conclusion, the investigational product (sachets containing an oral solution of hyaluronic acid, a mix of amino acids including proline, hydroxyl-proline and glutamine, and rice extract dispersed in a bioadhesive polymer matrix) is effective in relieving symptoms in patients with NERD without any safety concerns.

Author contributions: Davide Giuseppe Ribaldone: Conceptualization and writing – original draft and writing – review & editing the revised version. Rinaldo Pellicano: Conceptualization and writing – original draft and writing – review & editing the revised version. Divya Chandradhara: Conceptualization and data curation and formal analysis. Pendlimari Rajesh: Investigation. Marco Astegiano: Writing – original draft.
All authors approved the final version of the manuscript, including the authorship list.

References

1. Eusebi LH, Ratnakumaran R, Yuan Y, Solaymani-Dodaran M, Bazzoli F, Ford AC. Global prevalence of, and risk factors for, gastro-oesophageal reflux symptoms: a meta-analysis. *Gut* 2018;67:430–400.
2. Chuang TW, Chen SC, Chen KT. Current status of gastroesophageal reflux disease: Diagnosis and treatment. *Acta Gastro-Enterologica Belgica* 2017;80:396–404.
3. Richter JE, Rubenstein JH. Presentation and Epidemiology of Gastroesophageal

Reflux Disease. *Gastroenterology* 2018;154:267–276.

4. Katzka DA, Pandolfino JE, Kahrilas PJ. Phenotypes of Gastroesophageal Reflux Disease: Where Rome, Lyon, and Montreal Meet. *Clin Gastroenterol Hepatol* 2019 Jul 15. pii: S1542-3565(19)30748-7. doi: 10.1016/j.cgh.2019.07.015. [Epub ahead of print]
5. Herregods TVK, Bredenoord AJ, Smout AJPM. Pathophysiology of gastroesophageal reflux disease: New understanding in a new era. *Neurogastroenterology and Motility* 2015;27:1202–1213.
6. Savarino E, Zentilin P, Marabotto E, Bodini G, Della Coletta M, Frazzoni M, et al. A review of pharmacotherapy for treating gastroesophageal reflux disease (GERD). *Expert Opinion on Pharmacotherapy* 2017;18:1333–1343.
7. Galmiche JP, Clouse RE, Bálint A, Cook IJ, Kahrilas PJ, Paterson WG, et al. Functional esophageal disorders. *Gastroenterology* 2006;130:1459–1465.
8. Ribolsi M, Savarino E, De Bortoli N, Balestrieri P, Furnari M, Martinucci I, et al. Reflux pattern and role of impedance-pH variables in predicting PPI response in patients with suspected GERD-related chronic cough. *Aliment Pharmacol Ther* 2014;40:966–973.
9. De Bortoli N, Martinucci I, Savarino E, Bellini M, Bredenoord AJ, Franchi R, et al. Proton pump inhibitor responders who are not confirmed as GERD patients with impedance and pH monitoring: Who are they? *Neurogastroenterol Motil* 2014;26:28–35.
10. Rinsma NF, Farré R, Troost FJ, Elizalde M, Keszthelyi D, Helyes Z, et al.

Exploration of the Esophageal Mucosal Barrier in Non-Erosive Reflux Disease. *Int J Mol Sci* 2017;18(5). pii: E1091. doi: 10.3390/ijms18051091.

11. Silva RO, Oliveira FFB, Bingana RD, Arruda MO, Woodland P, Lee C, et al. A novel murine model of esophageal nonerosive reflux disease: from inflammation to impairment in mucosal integrity. *Am J Physiol Gastrointest Liver Physiol* 2017;312:G658–65.
12. Savarino V, Marabotto E, Zentilin P, Furnari M, Bodini G, De Maria C, et al. The appropriate use of proton-pump inhibitors. *Minerva Medica* 2018;109:386–399.
13. Tack J, Fass R. Review article: Approaches to endoscopic-negative reflux disease: Part of the GERD spectrum or a unique acid-related disorder? *Aliment Pharmacol Ther* 2004;19 Suppl 1:28-34.
14. Katz PO, Scheiman JM, Barkun AN. Review article: Acid-related disease - What are the unmet clinical needs? *Aliment Pharmacol Ther* 2006;23 Suppl 2:9-22.
15. Gupta N, Inadomi J, Sharma P. Perception about gastroesophageal reflux disease (GERD) and its impact on daily life in the general population: results from a large population based AGA survey. *Gastroenterology* 2012;142:S441–S441.
16. Savarino E, Zentilin P, Mastracci L, Dulbecco P, Marabotto E, Gemignani L, et al. Microscopic esophagitis distinguishes patients with non-erosive reflux disease from those with functional heartburn. *J Gastroenterol* 2013;48:473–482.
17. Tack J. Review article: Role of pepsin and bile in gastro-oesophageal reflux disease. *Aliment Pharmacol Ther* 2005;22 Suppl 1:48-54.
18. Suzuki T, Matsushima M, Masui A, Tsuda S, Imai J, Nakamura J, et al. Irsogladine

maleate and rabeprazole in non-erosive reflux disease: A double-blind, placebo-controlled study. *World J Gastroenterol* 2015;21:5023–5031.

19. Reimer C, Lødrup AB, Smith G, Wilkinson J, Bytzer P. Randomised clinical trial: alginate (Gaviscon Advance) vs. placebo as add-on therapy in reflux patients with inadequate response to a once daily proton pump inhibitor. *Aliment Pharmacol Ther* 2016;43:899–909.

20. Savarino V, Pace F, Scarpignato C, Esoxx Study Group. Randomised clinical trial: mucosal protection combined with acid suppression in the treatment of non-erosive reflux disease - efficacy of Esoxx, a hyaluronic acid-chondroitin sulphate based bioadhesive formulation. *Aliment Pharmacol Ther* 2017;45:631–642.

21. Palmieri B, Merighi A, Corbascio D, Rottigni V, Fistetto G, Esposito A. Fixed combination of hyaluronic acid and chondroitin-sulphate oral formulation in a randomized double blind, placebo controlled study for the treatment of symptoms in patients with non-erosive gastroesophageal reflux. *Eur Rev Med Pharmacol Sci* 2013;17:3272–3278.

22. Casale M, Moffa A, Sabatino L, Pace A, Oliveto G, Vitali M, et al. Hyaluronic Acid: Perspectives in Upper Aero-Digestive Tract. A Systematic Review. *PLoS One* 2015;10:e0130637.

23. Bhatia SJ, Makharia GK, Abraham P, Bhat N, Kumar A, Reddy DN, et al. Indian consensus on gastroesophageal reflux disease in adults: A position statement of the Indian Society of Gastroenterology. *Indian J Gastroenterol* 2019;38:411–440.

24. Aziz Q, Fass R, Gyawali CP, Miwa H, Pandolfino JE, Zerbib F. Functional

Esophageal Disorders. *Gastroenterology*. 2016 Feb 15. pii: S0016-5085(16)00178-5. doi: 10.1053/j.gastro.2016.02.012. [Epub ahead of print]

25. Kondo T, Miwa H. The Role of Esophageal Hypersensitivity in Functional Heartburn. *Journal of Clinical Gastroenterology* 2017;51:571–578.

26. Pace F, Santalucia F, Porro GB. Natural history of gastro-oesophageal reflux disease without oesophagitis. *Gut* 1991;32:845–848.

Table 1. Inclusion criteria

Diagnosis of NERD
Has experienced at least 3 episodes of moderate-severity heartburn during the 7-day run-in period.
Greater than 18 years of age.
Written informed consent.
Ability to follow a controlled diet (coffee and tea limited to not more than 2 cups per day; chocolate, alcoholic beverages and spices reduced as much as possible).

NERD: non-erosive reflux disease

Table 2. Exclusion criteria

Severe symptoms
Diagnosis of *H. pylori* infection.
Inability or unwillingness to return for study visits.
Suffers from other significant gastrointestinal diseases (including gastric and duodenal ulcers, infections or inflammatory conditions of the small or large intestine, and obstructions).
History of gastrointestinal surgery.
Malabsorption, Barrett oesophagus, oesophageal stricture, pyloric stenosis, or a history of GORD refractory to 2 months of therapy with either an H2RA or a PPI drug
The presence of comorbidities, including diabetes, metabolic disease, atopy, scleroderma, thyroidal disease, severe cardiovascular, renal, hepatic, pulmonary, or mental disorders, malignancy, HIV infection or any other immuno-compromised condition, chronic diarrhoea or irritable bowel syndrome.
Pregnant, lactating women.
History of alcohol or drug abuse within the past 5 years.
The presence of any condition which in the opinion of the Investigators may interfere with nutrient absorption, distribution, metabolism and excretion, including celiac disease, Crohn's disease, chronic pancreatitis, cystic fibrosis, lactase deficiency, lactose intolerance, biliary atresia, parasitic diseases, and diseases of the gallbladder, liver or pancreas.
Deteriorating health status at the time of enrolment, rapid weight loss, terminal disease.
Currently participating in or has participated in another clinical trial in the last 3 months

prior to the beginning of this study.

AST or ALT values ≥ 2.5 X ULN

Serum creatinine ≥ 1.5 mg/dL

Subject unwilling or unable to comply with the study procedures.

History of allergy to any component of the study product.

H. pylori: *Helicobacter pylori*; GORD: gastroesophageal reflux disease; H2RA: H2-receptor antagonist; PPI: proton-pump inhibitor; HIV: human immunodeficiency virus; AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ULN: Upper Limit of Normal

Table 3. Baseline characteristics of the 40 enrolled patients

	Test Product	Placebo	*P* value
Patient number	20	20	N/A
Age, geometric mean (95% CI, year)	33 (29.1 – 37.4)	36.5 (31 - 43)	0.31
Gender, M/F	15/5	13/7	0.73
BMI, median, (95% CI, Kg/cm^2)	26.7 (24.9 – 31.1)	24.7 (24 – 27.8)	0.10
Medication prior to study participation	Antacid (20/20)	Antacid (20/20)	1
NERD severity prior to study participation	Moderate (20/20)	Moderate (20/20)	1

N/A: not applicable; CI: confidence interval; F: female; M: male; BMI: Body mass index; NERD: non-erosive reflux disease

Table 4. Measure of quality of life using SF-36 questionnaire (median score)

	Test Product		*P* value	Placebo		*P* value
	Day 0	Day 14		Day 0	Day 14	
Physical activity	65 95% CI = 50.8 – 84.2	85 95% CI = 55 – 95	0.006	50 95% CI = 30 – 54.2	50 95% CI = 30 – 50	0.63
Physical role Limitations	50 95% CI = 0 – 100	100 95% CI = 100 – 100	0.001	0 95% CI = 0 – 95.8	0 95% CI = 0 – 100	ç
Reduction of 50% in physical pain	30 95% CI = 30 – 41	61 95% CI = 61 – 74	0.001	30 95% CI = 30 – 30	30 95% CI = 30 – 30	ç
General health	36 95% CI = 30 – 41.2	57 95% CI = 52 – 61	0.0008	30 95% CI = 30 – 35	35 95% CI = 30 – 41.7	0.06
Vitality	40 95% CI = 35.8 – 54.2	60 95% CI = 50 - 65	0.0007	40 95% CI = 35 – 44.2	40 95% CI = 30.8 - 45	0.85
Social activities	50	62	0.0006	50	50	0.84

	95% CI = 37 – 50	95% CI = 50 – 72.8		95% CI = 37- 50	95% CI= 37- 50	
Emotional role	33	100	0.001	0	33	ç
Limitations	95% CI = 0 – 100	95% CI = 100 – 100		95% CI = 0 – 94.2	95% CI = 0 – 100	
Mental health	44	60	0.005	48	40	0.03

SF: Short Form; CI: confidence interval; ç: inestimable *P* due to small sample size

Figure 1. Study flow-chart

Figure 2. Bar chart for proportion of patients with at least 3-point reduction of the total symptom score (TSS) (***P<0.001 vs placebo)

Figure 3. Bar chart of total symptom score during baseline (day 0), interim (day 7) and final (day 14) visit (***P<0.001 vs baseline; °°°P<0.001 vs placebo)

Figure 4. Bar chart for comparison of heartburn severity index at baseline (day 0), interim (day 7) and final (day 14) visit (***P<0.001 vs baseline; °°°P<0.001 vs placebo)