



Barreto R¹, Todai M², Kumar N³,
Hayakawa K², Marotta F^{4*}, Imaoka Y²
and Catanzaro R⁵

¹Gastrointestinal Endoscopy Department, National Institute of Medical Sciences and Nutrition, Salvador Zubiran, Mexico City, Mexico

²BioClinic Center & Corporation, Sendai, Japan

³Faculty of Applied Sciences, Shri Venkateshwara University, JP Nagar, Uttar Pradesh, India

⁴ReGenera Research Group for Aging-Intervention, Milano & San Babila Clinic, Vitality Therapeutics, CMB Unit, Milano, Italy

⁵Department of Clinical and Experimental Medicine, Section of Gastroenterology, University of Catania, Catania, Italy

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*Corresponding author: Francesco Marotta, ReGenera R&D International for Aging Intervention & San Babila Clinic, Milano, Italy, Tel: 393929165625; Fax: 39-02-407-7243; E-mail: fmarchimede@libero.it

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Research Article

More than a common acid buffering in the treatment of Non-Erosive GERD-like Clinical Spectrum: A Chios Masthia-based Formulation with (Cyto) Protecting Effect

Abstract

The aim of this study was to test the clinical and mucosal benefit of a natural gastro-oesophageal mucosal protector DOGDG-121 in non-erosive GERD comprising from NERD, to Functional Heartburn and Hypersensitive oesophagus while also ascertain the symptom-free duration in patients with prior symptomatic relapsing GERD. 68 consecutive adult patients, reporting mild heartburn symptoms two or more days a week or at least moderate symptoms more than once a week for at least six months duration were prospectively recruited. Inclusion criteria were: recurrent typical GERD symptoms, such as heartburn and/or acid regurgitation and a duration of symptoms >6 months. Patients were divided in two groups comparable as for age, gender, BMI and pHmetry profile. One group was given 1 sachet a day (2gr) of DOGDG-121 (chios masthia- and musa extract-based phyto compound, Reflumed, Named, Italy) quickly stirred in a small water quantity (≤ 30 ml) and swallowed at once, while the other was administered 5ml of a buffering antacid suspension (BAS) (Dried Aluminium Hydroxide Gel 230mg, Magnesium Hydroxide 200mg Simeticone 25mg) as positive control. At entry and after 12 weeks all patients underwent an ambulatory 24-Hours intraoesophageal pH monitoring. As compared to BAS, DOGDG-121 enabled a significantly lesser degree of microscopic oesophagitis, reduced gene expression of IL-8 and all Eotaxins tested ($p < 0.05$). This was also associated to a better gastrointestinal-related quality of life assessment ($p < 0.05$). This strongly suggested that, besides protection against acido-peptic mucosal injury per se, DOGDG-121 appears to offer a more significant effect on inflammatory mediators interfering with peripheral nociceptors, being amenable to safe, long-term cytoprotective use.

Introduction

Gastroesophageal-reflux disease (GERD) is one of the most common reported complaints in clinical practice and is an established cause of esophageal damage, ranging from non-erosive to erosive oesophagitis, intestinal metaplasia and cancerous transformation. By common understanding, most patients with gastro-oesophageal reflux disease (GERD) may be generally divided into two main categories, depending on the endoscopic evidence of erosive oesophagitis or non-erosive reflux disease (NERD). This latter cluster of patients may refer symptoms at time very suggestive of GERD but showing irrelevant visible mucosal finding when analyzed by upper gastrointestinal endoscopy, i.e. NERD [1]. As a matter of fact, NERD patients with mildly abnormal pH monitoring value (4.2% < total time < 7%) with their reportedly modest response to PPI once daily, behave like those suffering from

functional heartburn (normal pH test). This explains why the heterogeneity of this group has still undefined boundaries until finer diagnostic modalities are designed to unveil NERD in its full spectrum. However, around 20% of them who were initially responding to on-demand or intermittent PPI, over time will experience symptomatic relapses. This is why NERD is nowadays regarded as a chronic, relapsing illness liable to undergo periods of exacerbation with a relapse rate of 75% within 6 months if therapy is halted and, thus, needing long-term treatment strategy [2].

The stratified squamous epithelium of the oesophagus usually fully capable to offer an adequate protection against reflux. In response to a number of injuries, a damage to tight junctions, development of dilated intercellular spaces (DIS) and loss of squamous epithelium integrity arise with a pathologic exposure of the epithelial lining to gastric and duodenal juices

[3-9]. An increasing number of clinical and experimental models support the concept that more complex multifaceted mechanisms such as neural and inflammatory ones may be advocated for to explain the “chronicity” of the injury [4-7]. Such inflammatory mediators are released by squamous epithelial cells, immune cells, and a variety of stromal cells, the latter still under investigation due to their complex isolation and in vitro testing [8]. While this often prompts patients to frequent use of antacids, on the other hands, the search of preferably not chemical formulas with broader cytoprotective effect is of worthwhile interest. Besides other minor excipients, the main ingredients of a novel phytoformula, (DOGDG-121, ReFlumed, named, Italy) are chios masthia and banana. The healing properties of chios masthia against ulcer and gastritis date back in the middle 1980's [9,10] and a more recent study [11-17], suggested that arabinogalactan-proteins (AGP's) isolated by mastic could display some moderate anti-*H.pylori* activity, given his reported bactericidal activity in vitro [12]. However, this data has been questioned by conflicting data in in vivo tests [13]. As for its anti-inflammatory gastrointestinal properties, already in 2007, Kaliora et al. [14,15], demonstrated that mastic administration in patients with active Crohn's disease (CD) significantly reduced the CD activity index, plasma interleukin-6 and C-reactive protein levels while also recovering a normal total antioxidant potential. A properly done double-blind, placebo-controlled clinical study in this setting has been very recently published [16-22]. A minor biological ingredient of DOGDG-121, is a flavonoid-rich banana (i.e. herbaceous plants of the genus *Musa*) which has a long lasting traditional experience as to reduce gastric acidity with a propensity to coat the epithelial lining. Indeed, Lewis et al., has shown in experimental animal its gastric mucosa protection against aspirin [17,18], thanks to its Leucocyanidin with its hydroxyethylated and tetraallyl derivatives component. These data have been more recently confirmed by, albeit scanty and experimental, studies [19,20].

The aim of this study was to test the clinical and mucosal benefit of a natural gastro-oesophageal mucosal protector DOGDG-121 in non-erosive GERD comprising from NERD, to Functional Heartburn and Hypersensitive oesophagus while also ascertain the symptom-free duration in patients with prior symptomatic relapsing GERD.

Patients

68 consecutive adult patients, reporting mild heartburn symptoms two or more days a week or at least moderate symptoms more than once a week for at least six months duration were prospectively recruited. Inclusion criteria were: recurrent typical GERD symptoms, such as heartburn and/or acid regurgitation and a duration of symptoms >6 months.

Patients were divided in two groups comparable as for age, gender, BMI and pHmetry profile. One group was given 1 sachet a day (2gr) of DOGDG-121 quickly stirred in a small water quantity (≤ 30 ml) and swallowed at once (prof. Marotta's modification as compared to manufacturer's instruction), while the other was administered as 5ml of a common buffering antacid suspension (BAS) (Dried Aluminium Hydroxide Gel

230mg, Magnesium Hydroxide 200mg Simeticone 25mg) as positive control.

In those cases of patients experiencing higher symptom scoring during the initial part of the study, a double dosage was allowed and patients were maintained within the study.

A third group of 20 dyspeptic patients, age-, BMI- and gender-matched with the above two groups, confirmed as non-refluxers nor referring any NERD-like symptom represented a control group. These subjects while undergoing a screening upper gastrointestinal endoscopy, had 4 biopsies (1 for histology and 3 for cytokine markers) were taken. The control rationale was based on the need to obtain a cytokine gene expression with a nominal “normal” profile to refer to.

Exclusion criteria: active oesophagitis, present or prior Barrett's oesophagus, gastric dysplasia, prior GI cancer wherever located, prior cancer of any other organ, GI tract surgery (with the exception of appendectomy), NSAIDs or aspirin users, active drinkers and consumption of more than three cups of tea, coffee or cola. Drugs like histamine-2 receptor antagonist, prokinetics, proton pump inhibitors (PPI) and anticholinergic agents within the last three weeks of the study and history of intolerance or allergy to any of the ingredients of these treatments. Persistent utilization of drugs other than those for diabetes mellitus and high blood pressure were also considered as an exclusion criteria. Recruited subjects were asked not to use, unless specifically prescribed by physician, inhaled, topical steroids during the study period.

Ethics the research was in accordance with the Declaration of Helsinki. All the participants gave written informed consent before the participation and local ethical committee approved the study. The protocol registered as 26/2018 had been duly evaluated by ethical committee in Italy and Mexico.

Methods

Ambulatory 24-Hours intraesophageal pH monitoring

A water perfused esophageal manometry enabled to precisely locate the lower esophageal sphincter (LES). Based on this evaluation, a pH Monitoring Device (Digitrapper Mark III system; Synetics Medical AB, Stockholm, Sweden) equipped with amonocrystalline antimony pH catheter with 2 sensors was employed. Reflux events were defined when pH dropped below 4 to later returning to pH above it. Total reflux time (Time for pH less than 4.0), count of reflux periods, the duration for the longest reflux event and DeMeester grading scored as a whole and analyzing separately the supine and upright position.

Endoscopic evaluation and oesophageal biopsy samples evaluation

The squamocolumnar junction was defined as the border between the gastric glandular and esophageal squamous epithelium and it roughly corresponded to the proximal edge of the gastric folds. The distal portion of the esophagus was carefully evaluated in order to determine the presence of mucosal injury.

A total of five biopsies from each individual, 2 for routine histological evaluation and 3 for analysis of MCP-1, IL-8, Eotaxin-1, Eotaxin-2 and Eotaxin-3 gene expression, were taken at 3–5 cm above the squamocolumnar junction in NERD patients. Fresh tissues for inflammatory gene biomarker evaluation were maintained at -80°C until measurement of mRNA. Two biopsy samples were taken from the antrum to test for HP (by modified Giemsa staining), and each time of sampling the endoscope channel was flushed by 30 cc sterile 0.9% saline. Histological evaluation was independently performed by two expert pathologists in a blinded fashion with an overall very good inter observer agreement ($K = 0.81$). Any divergent opinion in this estimation was discussed in depth to reach a mutually agreed scoring. Biopsy specimens were oriented over cellulose acetate papers, fixed in 10% buffered formalin, and embedded in paraffin blocks. Four sections of each sample were examined and the most appropriate sample was carefully chosen each time for the histological assessment.

Esophageal biopsy samples evaluation

Squamous oesophageal samples were examined for oesophagitis. The degree of inflammatory changes was evaluated in formalin-fixed sections stained with H&E and Alcian blue using the updated Sydney system. A semiquantitative calculation of basal cell hyperplasia (BCH), papillae elongation (PE) and dilatation of intercellular spaces (DIS), scored as 0 (if absent), 1 (if of mild degree), and 2 (if of more severe entity) was performed over the hematoxylin–eosin-stained slides and using computer-assisted morphometry ($\times 800$ magnification) (cellSens; Olympus, Tokyo, Japan). Either the thickness of basal cell and papillae length were assessed as a percentage of the whole epithelium as well as, possible intraepithelial infiltration of eosinophils (IE) and erosions was assessed. The mean of the most infiltrated three high power fields ($40\times$) were calculated, given normal values as follows: basal cell $<15\%$ and length of papillae $<66\%$. When lesions were not homogeneously distributed in a given sample, the most severe abnormality was taken into consideration.

RNA isolation real-time reverse transcription polymerase chain reaction

Biopsies were immediately transferred to 2 ml of RNA solution, and total RNA was isolated by a RNA easy Qiagen Mini Kit (Qiagen, Valencia, CA) following manufacturer's protocol. To eliminate DNA contamination. One μg of total RNA was treated by DNAase I according to the product manual. RNA was reversely transcribed and subjected to real time PCR by using Gene Amp Gold RNA PCR Reagent Kit and Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) respectively. The CFX96 (Bio-Rad) using TaqMan gene expression assays from the Applied Biosystems (Life Technologies, Foster City, CA, USA). Identical threshold was applied to each gene of interest. In order to guarantee that the desired amplicon was detected, each time the dissociation curve was run after the real time PCR. The target sequences for qPCR were as follows: monocyte chemoattractant protein-1 (MCP-1) (F-5'GATCTCAGTGCAGAGGCTCG3'; R-5'TGCTTGTCCAGGTGGTCCA T3', interleukin 8 (IL-8) F-5'GCAGCCTTCCTGATTTCTGCAGCTC3';

R-5'ACTTCTCCACAACCCCTC, Eotaxin 1: forward, 5-CTCGCTGGGC-CAGCTTCTGTG-3; reverse, 5-GGCTTTGGAGTTGGAGATTTT-GG-3; Eotaxin-2: forward, 5-CACATCATCCCTACGGGCTCT-3 reverse, 5-GGTTGCCAGGATATCTCTGGACAGGG-3; eotaxin-3 pGL3-EOT3M1, 5'-TGTTCCCAACCACAGAATAGTCTGGAATT-GTTTT CAGGGCCGT-3_ and 5_-GAGACGGCCCTGAAAAAATTC-CAGACTATTCTGTG GTTGGGA-3. glyceraldehyde 3-phosphate dehydrogenase GAPDH, F-5'CCGGAAACTGTGGCGTGATGG3'; R-5'AGGTGGAGGAGTGGGTGTCGCTGTT3', the latter being used as an endogenous control, based on which the Ct value were normalized and identical threshold was applied to each gene of interest. mRNA relative expression levels were then calculated using the delta cycle threshold (Ct) method. All standards and samples were subjected to PCR simultaneously and in duplicate. The principles of methodologies for the above biomarkers are already been reliably reported in the past [21–23].

Western blot analysis

Same amounts of total proteins from samples coming from different treatments, as reported in the literature [24], were parted on 15% SDS-PAGE and shifted onto polyvinylidene difluoride membranes. Blots were then incubated with adequate HRP-linked secondary antibodies to be then developed with ECL Western blot substrate.

Primary outcome: To check any potential benefit of this compound on microscopic mucosal inflammation, ultrastructural morphology. And gene expression of mucosal inflammatory markers.

Secondary outcome: symptoms score (heartburn, belching, epigastric pain, gastric bloating, slow digestion, nausea), symptom free duration in those patients with prior documented history of more than 3 episodes of heartburn per week.

Secondary efficacy endpoints: There were four secondary endpoints which were studied in this investigation: (1) the change in the severity of reflux symptoms including heartburn, regurgitation, epigastric soreness, nausea, vomiting and belching by VAS at the 4th week and 12th week of treatment as compared to baseline; (2) the change in the quality of life as evaluated from doctor's point of view together with the patient. During the entire study period, each patient had to record the frequency (number of episodes per day) on the patient's diary. At least a 4-day record in a week was requested as minimum mandatory data to carry out this evaluation.

As a further endpoint (3rd), it was applied an extended QOL assessment, i.e. Quality of Life in Reflux and Dyspepsia (QOLRAD) which is a very validated tool in patients presenting with upper gastrointestinal symptoms [25,26]. QOLRAD contains 25 questions addressing concerns associated with gastrointestinal symptoms. The questions are rated on a seven-grade Likert scale; the lower the value, the more severe the impact on daily functions. The questions are categorized into five areas: emotional distress (six questions), sleep disturbance (five questions), vitality (three questions), food/drink problems (six questions) and physical/social functioning (five questions) and the total QOLRAD score is calculated as the mean of each value.

Statistical analysis

Comparisons of frequency employed the χ^2 test of Fisher's exact test, the test of linear trend for the ordered variables, Student's *t*-test of non-parametric Wilcoxon test and variance analysis for the repeated VAS, all adjusted to the initial values. Two-sample *t* test was used to compare the continuous data such as height, weight, age, the number of heartburn, the number of sleeping disturbance etc., and the 95%CI for the difference were calculated. Statistical significance was assessed at the 5% level. The principle analysis was carried out on an ITT basis.

Results

Gastroesophageal Symptoms evaluation. Both treatments brought about a statistically significant improvement of heartburn already starting after two weeks treatment and of comparatively extent ($p < 0.05$, Figure 1). However, starting from the 4th week observation, DOGDG-121 enabled a significantly better heartburn relief ($p < 0.05$ vs control compound, Figure 1). Indeed, along this observation period, patients taken as overall, rated their heartburn less than as half as it was at the entry and this was more evident than in BAS-treated ones ($p < 0.05$). This difference was particularly evident when specifically analyzing the "confirmed NERD" cases ($p < 0.01$, data not shown). When evaluating the health status by applying the wider gastrointestinal-centered (GIC) quality of life assessment (GIC-QUOLRAD), the overall sum of the questionnaire showed the significant superiority of DOGDG-121 over the BAS compound ($p < 0.05$, Figure 2) owing to higher values of three parameters over five, i.e. physical and social functioning, food and drinks limits (breakdown data not shown).

This determined a significant shift of DOGDG-121-treated subjects towards pauci-symptomatic variant of the syndrome with lower frequency of symptomatic flare ups ($p < 0.01$ vs baseline and BAS treatment, data not shown).

pH-metry profile subtypes

As shown in table 1, 24h oesophageal pH monitoring prior

CHANGE IN THE VAS OF HEARTBURN: AT 12th WEEK

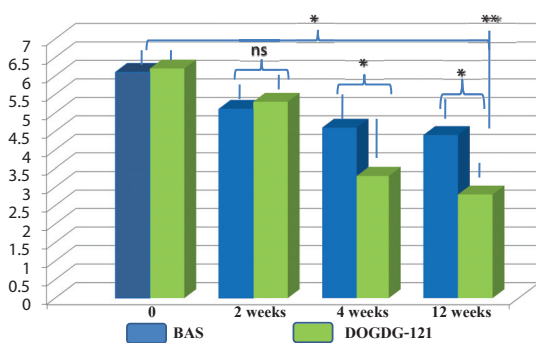


Figure 1: Changes in the heartburn score as assessed using a visual analog scale (VAS). Solid green bars represent the patients treated with d DOGDG-121 and blue bars represent the control BAS-treated group. * $p < 0.05$ vs. baseline and BAS con-trol; § $P < 0.05$ vs. baseline with no difference between DOGDG-121 and BAS-treated control; ** $p < 0.01$ vs. baseline.

CHANGE IN THE GASTROINTESTINAL SYMPTOMS CENTERED QUALITY OF LIFE IN NERD PATIENTS

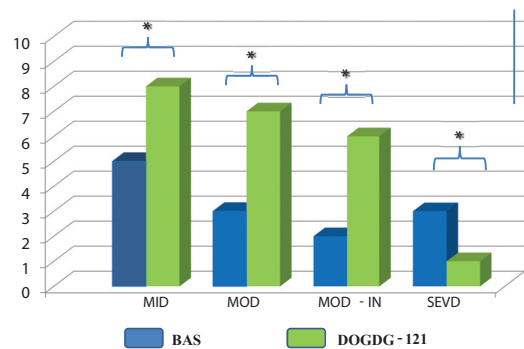


Figure 2: Change in the gastrointestinal symptom centered quality of life in NERD patients. Green bars represent baseline, solid grey bars represent patients treated with DOGDG-121 and blue bars represent the control group treated with BAS. NERD, non-esophagitis reflux disease; NS, no symptoms; MID, mild dyscomfort MOD moderate dyscomfort that does not interfere with normal life; MOD IN: moderate dyscomfort interfering with normal life; SEVD: severe dyscomfort. * $p < 0.05$ vs. baseline and BAS-treated control; ** $p < 0.05$ vs. baseline.

Table 1: Clinical And Ph-Metry Profile Of GERD-Like Symptomatic Patients.

Categories	Confirmed NERD	Functional heartburn	Hypersensitive Oesophagus	p value
n. of patients	20	12	26	
Mean age	57.6 ± 11.2	59.6 ± 16.7	53.7 ± 13.7	ns
Mean BMI kg/m ²	25	24	26	ns
Hiatal hernia	13/20 (65%)*	2/12 (16%)	9/26 (34%)	<0.05
PPI Responders	15/20 (75%)*	2/12 (16%)	6/26 (23%)	<0.05
Acid exposure (%)	11.3 ± 3.6*	0.6 ± 0.5	0.9 ± 0.3	<0.01
Total n. of reflux	46.7 ± 25.9	41.7 ± 16.7	34.4 ± 14.2	ns

to setting the two comparable groups was composed by "true" NERD, Functional heartburn and hypersensitive oesophagus in the percentage of, 34.4%, 20.6% and 44.8%, respectively. The first group was understandably the one with a significantly higher acid exposure ($p < 0.005$) and a trend higher number of reflux episodes. This group was also the one reporting better, albeit uncompleted, prior PPI response (75%) while Functional heartburn patients were the poorer PPI responders. The confirmed NERD cases were also diagnosed hiatal hernia at higher incidence as compared to the other groups ($p < 0.05$).

Microscopic evaluation of inflammation

When looking at the presence of microscopic oesophagitis, it appeared that also on GERD or NERD subjects had, albeit negligible, signs of microscopic oesophagitis. However, these features were significantly more detected in functional heartburn, hypersensitive oesophagus and NERD, along a gradually increasing gradient with statistical significance among each group and the one above ($p < 0.05$, Figure 3). Among the specific histologic parameters (Figure 4), it appeared that basal cell hyperplasia, papillary elongation and intra-epithelial eosinophils findings were comparable between controls and patients with Functional Heartburn, whereas either Hypersensitive Oesophagus and NERD group showed significantly elevated values in all considered parameters ($p < 0.01$). Patients with Functional Heartburn showed a significantly higher value of IS as compared to control.

When taken overall either the microscopic histological abnormalities and the GERD/NERD-like patients subtypes, it appeared that, as compared to BAS, DOGDG-121 yielded a statistically significant histological improvement (Figure 5, $p < 0.05$). However, this was partly complete and still more represented than in control subjects (26% vs 8%, $p < 0.05$).

Gene expression study

As shown in table 2, MCP-1 gene expression was comparable to values recorded in healthy control. All other tested biomarkers had a varying degree of significant overexpression above healthy subjects (data not shown, $p < 0.05$). However, only Eotaxin-1 gene expression was significantly higher in “confirmed NERD” subgroup whereas none of the other tested biomarkers had a discriminatory differential diagnostic power.

Values represent a ratio as compared to healthy control given the value of 1. Except MCP-1 values, all the other baseline values were significantly higher than healthy control ($p < 0.05$).

Gene expression of inflammatory markers detected in lower oesophageal mucosa showed that at 12 weeks observation, unlike BAS, DOGDG-121 significantly reduced the overexpression of interleukin-8 ($p < 0.05$, Figure 6). No MCP-1 mRNA changes occurred the studied patients nor any variation upon different treatment (ns, Figure 7). All three Eotaxins were significantly overexpressed ranging from 20% to 360% in comparison to the default baseline value assigned to healthy subjects (Figures 8-10). Either BAS and DOGDG-121 determined a beneficial downregulating effect of Eotaxin-1 and Eotaxin-3 ($p < 0.05$ vs baseline) but DOGDG-121 showed to exert a significantly better response ($p < 0.05$ vs BAS). The significant overexpression of Eotaxin-2 over healthy subjects was not affected by BAS but remarkably decreased when tested in patients treated with DOGDG-121 ($p < 0.05$ vs BAS).

Table 2: Oesophageal Inflammatory Markers Gene Expression In Ph-Metry Profile of GERD-Like Symptomatic Patients.

Biomarkers	Confirmed NERD	Functional heartburn	Hypersensitive Oesophagus	p value
MCP-1	1.5 ± 0.2	1.2 ± 0.3	1.1 ± 0.1	0.87
IL-8	1.2 ± 0.2	1.1 ± 0.5	1.4 ± 0.7	0.48
Eotaxin-1	3.3 ± 0.6	2.6 ± 0.9	2.3 ± 0.4	<0.005
Eotaxin-2	1.7 ± 0.9	1.3 ± 0.3	1.3 ± 0.6	0.54
Eotaxin-3	4.1 ± 1.1	3.8 ± 0.8	4.3 ± 1.2	0.09

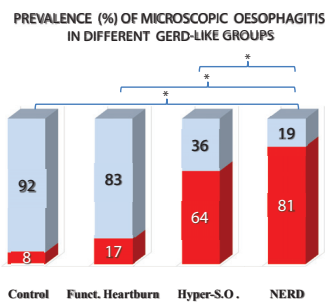


Figure 3: Prevalence of microscopic oesophagitis (in red) in different groups of suspected refluxers. As compared to control, each group showed a statistically higher prevalence of histological damage ($p < 0.05$). Funct. Heartburn: functional heartburn; Hyper-S.O.: hypersensitive oesophagus. Either Hyper-S.O. and “true” NERD showed an histological damage at a higher prevalence as compared to Funct. Heartburn ($p < 0.05$). This was more prevalent in true NERD vs Hyper-S.O. ($p < 0.05$).

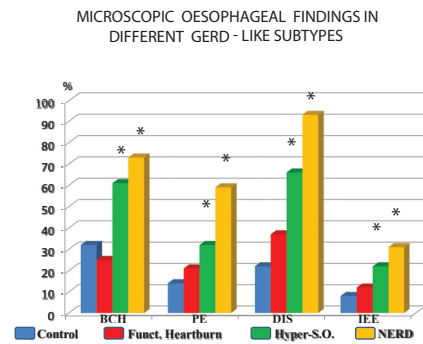


Figure 4: Distribution of the different microscopic histological parameters as follows: basal cell hyperplasia (BCH), papillae elongation (PE), dilatation of intercellular spaces (DIS) and intra-epithelial eosinophils (IEE) among different GERD-like subtypes. Functional heartburn and “true” NERD were the subtypes showing a statistically significant higher prevalence of all the above parameters ($p < 0.05$ vs control).

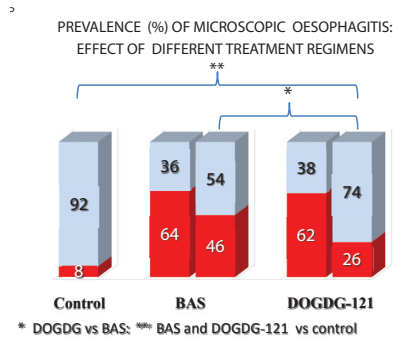


Figure 5: Effect of different treatments regimens on microscopic oesophagitis. While BAS treatment yielded a not significant decrease of microscopic damage, DOGDG-121 brought about a partial but significant improvement as compared to baseline data and to post-treatment obtained in BAS group ($p < 0.05$).

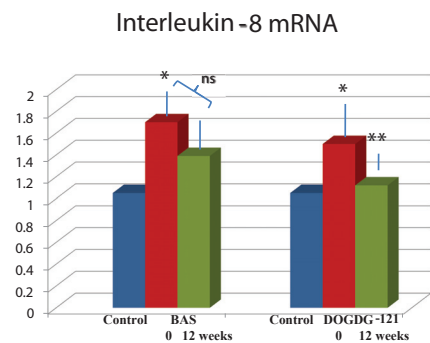


Figure 6: Effect of different treatments on Interleukin-8 mRNA, measured as a ratio vs GAPDH as an endogenous control at baseline (red bar) and 12 weeks (green bar).

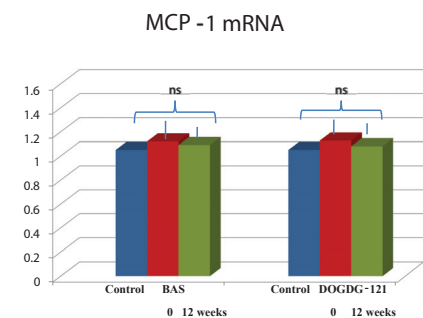


Figure 7: Effect of different treatments on Interleukin-8 mRNA, measured as a ratio vs GAPDH as an endogenous control and normalized to healthy control value at baseline (red bar) and 12 weeks (green bar). * $p < 0.05$ vs control; ** $p < 0.05$ vs post-treatment BAS value

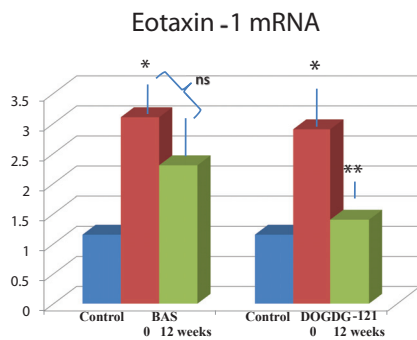


Figure 8: Effect of different treatments on Eotaxin 1, measured as a ratio vs GAPDH as an endogenous control and normalized to healthy control value at baseline (red bar) and 12 weeks (green bar). * $p < 0.05$ vs control; § $p < 0.05$ vs baseline; ** $p < 0.05$ vs post-treatment BAS value.

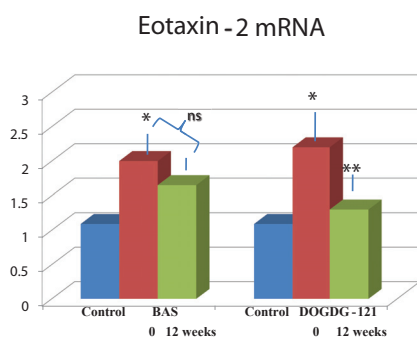


Figure 9: Effect of different treatments on Eotaxin 2, measured as a ratio vs GAPDH as an endogenous control at baseline (red bar) and 12 weeks (green bar). * $p < 0.05$ vs control; ** $p < 0.05$ vs post-treatment BAS value.

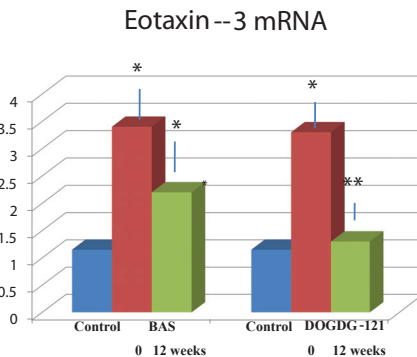


Figure 10: Effect of different treatments on Eotaxin 3, measured as a ratio vs GAPDH as an endogenous control at baseline (red bar) and 12 weeks (green bar). * $p < 0.05$ vs control; § $p < 0.05$ vs baseline; ** $p < 0.05$ vs post-treatment BAS value.

Discussion

The Rome II Committee for Functional Esophageal Disorders [6,27], in the lack of pathological gastroesophageal reflux or dysmotility, regarded these subjects as having functional heartburn, whereas the Rome III Committee merged the hypersensitive oesophagus cases and those with negative symptom association but responsive to PPI treatment [28]. This position was further confirmed by Rome IV Committee 10 years later [8,29]. Interestingly, the PPI (at standard dose) therapeutic response rate in NERD patients is quite suboptimal averaging 20%–30% less than what obtained in subjects with erosive esophagitis [30]. pHmetry monitoring has helped unveiling that among NERD patients, the one better responding

to PPI are those in whom the characteristic symptoms better parallel the extent of acid exposure of lower oesophageal mucosa. However, a relevant number of NERD patients show only a limited acid over-exposure and this clarifies the modest symptomatic benefit to PPI as compared to those patients with erosive esophagitis [31–33]. What seems evident so far is that most of NERD patients will never develop erosive esophagitis or Barrett's esophagus [32]. Indeed, growing evidence shows that NERD patients are characterized by enhanced esophageal sensitivity to chemical and mechanical stimuli caused by heightened excitability of visceral sensory neurons [33]. This phenomenon is possibly associated with overexpression of acid-sensing receptors in the epithelial layer and in the afferent fibers within the *lamina propria* [34]. Most individuals with mild GERD tend to self-treat themselves with over-the-counter medications [6] only seeking professional help if symptoms persist or complications arise but this does not necessarily help unfolding the phenomenon nor making sure about the appropriateness of the treatment. A wide range of tablet, liquid and gel formulations is available for the treatment of GERD, and antacids being amongst the most used formulations. From a practical point of view, using the least powerful medication at the lowest dosage possible in order to control symptoms is the optimal way for improving disease management and quality of life. As a matter of fact. Our study showed that DOGDG-121 controlled heartburn significantly better than BAS and this clinical parameter mostly affected also the quality of life of patients. This was associated to better histological findings, whenever present. Indeed, it was of interested that even patients without “confirmed NERD” and negligible histological damage who showed, as a whole, significantly lesser acid exposure and reported a past history of being poorer PPI response, received a measurable benefit from DOGDG-121 treatment. In fact, either acid and other luminal small molecules causing even mild injury, may penetrate into the epithelium and trigger specific embedded pain-sensitive nerve endings causing discomforts such as heartburn.

These data are in accordance with novel understanding of the phenomenon reported in recent years where the traditional model of the pathogenesis of reflux esophagitis and heart burn is challenged [35]. In this study Miwa et al., found no statistically significant differences between histological markers of esophagitis, the percentage of acid exposure of the lower oesophagus, or the number of gastric acid reflux episodes between reflux oesophagitis and NERD patients. Interestingly, they found out a significant increase in the mean number of nerve fibers in the NERD group compared to the reflux oesophagitis. The suspect that those PPI resistant cases bearing abnormal DIS may be linked to the 2-fold increase in neuronal immunostaining lead to suggest that, besides acid-peptic injury, cytoprotective action on nociceptive pathways should also be pursued. In this respect, one can envisage that DOGDG-121 offered more than a simple acid buffering property but, rather, a better effects on inflammatory mediators interfering with peripheral nociceptors.

In our study we found a limited eosinophils infiltration which is known to be a peculiar feature of reflux disease causing

acid-related damage and release of chemotactic cytokines. Indeed, MCP-1 was not significantly altered. However, other relevant chemokines such as IL-8 and Eotaxins were substantially downregulated by DOGDG-121 and significantly more than the antacid buffer. This holds important considering that mucosal IL-8 levels have been reported to be associated with endoscopic severity and risk of disease relapse [2], while our DOGDG-121-treated patients showed a negligible flare up rate. At the same time, the anti-dyspeptic effect of Chios Masthia can be advocated for its contribution in improving the overall gastrointestinal-related quality of life. This latter activity, together with the above-mentioned potent anti-inflammatory properties [10,11,18-20,31] partly shared also by *Musa paradisiaca* [17,20,36] may prove to provide a valid alternative to current therapies, while also envisaging a safe long-term use for its multifaceted cytoprotective effect.

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