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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

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Abstract. – BACKGROUND: Proton pump inhibitors (PPIs) are a major breakthrough in the medical management of gastroesophageal reflux disease (GERD). In several patients with non erosive reflux disease symptoms (NERD) the response to PPIs is partial or limited and symptoms relief needs the administration of additional medications.

AIM: The aim of this study was to evaluate the effect of a new medical device, based on an oral fixed combination of hyaluronic acid and chondroitin-sulphate (HA+CS), in a bioadhesive carrier, in adults with symptoms of non erosive gastroesophageal reflux and with a low response to PPIs.

PATIENTS AND METHODS: Twenty patients who had experienced heartburn and/or acid regurgitation for at least 3 days during a 7 day run-in period, without endoscopic mucosal breaks, were randomized in a double blind crossover study to receive four daily doses of a fixed oral combination of HA+CS and placebo for 14 days. Relief of cardinal symptoms of GERD was evaluated at the end of each period.

RESULTS: A significant greater Sum of Symptoms Intensity Difference, compared to placebo, was observed after HA+CS treatment (–2.7 vs 0.5 – $p < 0.01$), being both heartburn (–1.6 vs 0.5 – $p < 0.03$) and acid regurgitation (–1.1 vs 0.1 – $p < 0.03$) significantly improved by the medical device. A speed of action ≤ 30 min was significantly more frequently reported by patients during HA+CS administration than with placebo (60% vs 30% – $p = 0.05$). Total disappearance of symptoms was observed in 50% of the patients compared to 10% during placebo administration ($p = 0.01$ between group comparison).

CONCLUSIONS: A fixed combination of HA+CS has demonstrated to be effective in gastroesophageal reflux control, with a rapid onset of action.

Key Words:
Gastroesophageal reflux disease, Cross-over design, Heartburn, Acid regurgitation, Hyaluronic acid, Chondroitin-sulphate.

Introduction

Gastroesophageal reflux disease (GERD), caused by retrograde flux of gastric contents into the esophagus, is the most common digestive disease in Western Countries, with an estimated prevalence of 20% to 40% of adults, presenting troublesome heartburn and regurgitation[1-4]. At endoscopy, 60% of patients with typical GERD symptoms do not present evidence of mucosal damage (non-erosive reflux disease, NERD)[5]. NERD patients have either abnormal acid exposure in the 24 hours or strict relationship with acid reflux episodes[6]. Recent studies have emphasized that non-acidic reflux may also contribute to symptoms generation[7-9].

The current medical management of GERD is based on the administration of acid secretion inhibitors such as proton pump inhibitors (PPIs)[10-13]. Although PPIs are undoubtedly effective in the treatment of GERD patients, in more than 30% of patients, PPI therapy fails to completely resolve symptoms. This number is even higher in NERD patients where failure rates > 40% have been reported[14-15]. Despite improved compliance and proper time intake of medication, twice daily dosing of PPIs, reflux symptoms can persist, new symptoms can occur or be unmasked with esophagitis as final complication. Therefore, a real medical need is

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represented by new treatment option for NERD patients, expecially when complete resolution of symptoms is considered as endpoint.

A new completely natural medical device based on a combination of hyaluronic acid and chondroitin-sulphate (HA+CS) in a bioadhesive carrier (Lutrol®) may constitute a modern approach to GERD cardinal symptoms relief.

Hyaluronic acid, mainly present in the extracellular matrix of soft connective tissues, is involved in several key processes such as control of epithelial cells turnover, favouring re-epithelization and mucosal hydratation in ulcer healing[16].

Chondroitin-sulphate is a safe glycosaminoglycan, main component of mucous secretion of parietal cells, able to inhibit pepsin induced damage of the gastroduodenal mucosa. It may be of benefit in disease where inflammation is an essential marker[17-18]. The bioadhesive carrier is effective in coating the esophageal epithelium as long as possible with these natural compounds, acting as a buffering agent to form a barrier for the acidity of the gastric fluid and to prolong the action on esophageal mucosa[19].

We originally conceived this natural compounds association, on the work hypothesis of an empyrical galenic formula, for the anedoctical treatment of some selected drug resistant acid and alkaline gastritis patients, at the "second opinion medical office"; as a matter of fact, adjusting the dosage and the administration schedule, both active substances could improve the balance between offensive and defensive mechanism at esophageal mucosa level and reduce the dilated intercellular spaces avoiding H^+penetration and consequent nervous fiber stimulation, responsible of typical symptoms[20-21]. On the basis of the effective clinical improvements achieved, we planned to extend the study, accordingly, to a wider range of esophagitis patients.

Patients and Methods

This randomized, double blind, placebo-controlled, two-way cross over study was conducted in accordance with International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use and the Declaration of Helsinki. An informed consent was signed by patients with the formal acceptance to self-administration of the medical device compound under investigation and relative placebo.

A total of 33 patients were screened, but only 20 patients with non-erosive gastroesophageal reflux symptoms were enrolled in the study.

20 Patients, both sex, aged ≥ 18 years, attended two screening visits (at least 1 week apart). During the first screening visit (V1), diagnosis of GERD was established or confirmed. An endoscopy[22] to rule out any existing esophageal erosion and a ^{13}C urea breath test[23] to exclude *Helicobacter Pylori* presence were performed in all patients. Heartburn, acid regurgitation and relative severity for at least 3 days in the past week, inadequately controlled by PPIs, antiH2 and antacids were checked, for a requested Sum of Symptoms Severity Intensity (SSSI) ≥ 3. The second screening visit (V2) was planned to confirm 7 days after, the clinical situation, in spite of the maintenance of a constant GERD therapy. Eligible subjects were randomized to one of the two treatment periods and received the first study drug to be assumed for the following 14 days. Patients could received one spoon of syrup containing hyaluronic acid + chondroitin-sulphate or placebo, administered, far from meals, every 8 hours during daytime and two spoons at bedtime. Patients have to record on a specific daily card[24] the number of administered doses, daily intensity of symptoms, time elapsed to onset of action, duration of the effect , antacids as needed use. Intensity of heartburn and acid regurgitation at awakening and/or at bedtime, were daily recorded by the patients, using a 4-point rating scale as follows (0 = absence of symptom, 1 = minimal awareness of symptom, easily tolerated 2 = awareness of symptom which is bothersome but tolerable without impairment of sleep or daily living, possible use of antacids 3 = symptom hard to be tolerated interfering with daily activities and/or sleeping, recurrent use of antacids.

At V3 (end of the first 2-week treatment period) the subjects had to return the compiled daily card and were interviewed on emergent adverse effects (AEs), drug acceptability and patient's compliance.

After a wash out period of at least 7 days, patients were invited to return to the centre for V4 in order to repeat baseline evaluations and receive the drug for a second period of 14 days. During the final visit (V5) the same data collection performed at V3 was completed.

Proton pump inhibitors and H2-receptor antagonists, at a constant dose in the past week, were maintained at the same dosage over all the study period. Antacids were permitted on an as needed

base. Theophylline or xantine derivatives (coffees, tea) had to be limited to not more than two cups per day. Patients with life-threating concomitant disease were excluded.

The primary efficacy variables were the Sum of Symptoms Score Intensity (SSSI) over the 14-day treatment period, also expressed as Sum of Symptoms Intensity Difference (SSID), the difference obtained by subtracting SSSI at each time point from baseline value. Other efficacy variables were: speed of action, defined as time elapsed from drug intake to complete symptoms disappearance and classified according to Chevrel[25] as < 15 min, 15-30 min, > 30 min. For patients without any benefit, a maximum of 90 min was considered. Duration of action was defined as time elapsed from complete symptoms disappearance to symptoms reappearance. Any clinically relevant change in weekly use of antacids was considered. Tolerability and safety evaluation were based on AEs reporting, patient's syrup acceptance based on taste, swallowing difficulties due to viscosity.

Statistical Analysis

The primary variables SSSI and SSID, were analyzed using repeated-measures ANOVA with time points as factor within subjects. Speed and duration of action were analyzed using the Mann-Whitney U test. The rates of patients free from symptoms, reporting a rapid onset of action and AEs (coded by MedDRAsystem organ class) were analyzed by chi-square test or Fisher exact test. $p < 0.05$ was considered statistically signifcant.

Results

A total of 20 patients, 17 males and 3 females, mean age 55 ± 18 years (range 37-74), mean body mass index of 28.3 ± 5 kg/m^2 (20% BMI ≥ 30 kg/m^2) completed both randomized treatment periods. Patients had suffered from GERD for a mean of 4.5 ± 3.4 years and most of them were currently on treatment with PPIs alone or PPIs plus antacids. Baseline clinical characteristics were similar and comparable at the start of each treatment phase. Sum of Symptoms Severity Score intensity (SSSI) was 4.57 ± 1.6 at randomization and patients reported a mean of 4.2 days of heartburn episodes for a mean severity score of 2.67 ± 1.2 in the week preceding inclusion. Concomitant acid regurgitation was present in 18 subjects with a mean severity score was 1.87 ± 1.4 (Table I).

Table I. Demographic and clinical features of GERD patients.

Age, years: mean (SD)	55 ± 18
Range	37-74
Males: N (%)	17 (85)
BMI, kg/m^2: mean (SD)	28.3 (5.0)
– BMI ≥ 30 kg/m^2: N (%)	4 (20.0)
GERD, time to diagnosis, years, mean (SD)	4.5 (3.4)
Sum of Symptoms Severity Score index (SSSI): mean (SD)	4.57 (1.6)
Heartburn	
– Severity score: mean (SD)	2.67 (1.2)
– Number of days of episodes per week: mean (SD)	4.2. (1.1)
Acid regurgitation intensity: mean (SD)	1.87 (1.4)
GERD treatment: N (%)	
Proton pump inhibitor (PPI)	11 (55)
PPI + antacids	4 (20)
H2 receptor antagonist + antacids	2 (10)
Antacids	3 (15)

At the end of each treatment period, all patients exhibited a satisfactory compliance to drugs scheduled regimen, independently from randomized sequence (96% for H+C, 92% for placebo).

SSSI absolute value at the end of HA + CS treatment was significantly lower compared to placebo treatment (from 4.5 ± 1.4 to 1.83 ± 2.2 and from 4.0 ± 2.1 to 3.4 ± 1.9 respectively, $p < 0.01$ – Figure 1), whatever was the randomized sequence. Concomitantly a statistically significant Sum of Symptoms Intensity Difference (SSID) was detected (–2.7 ± 1.4 vs -0.6 ± 2.1 $p <$

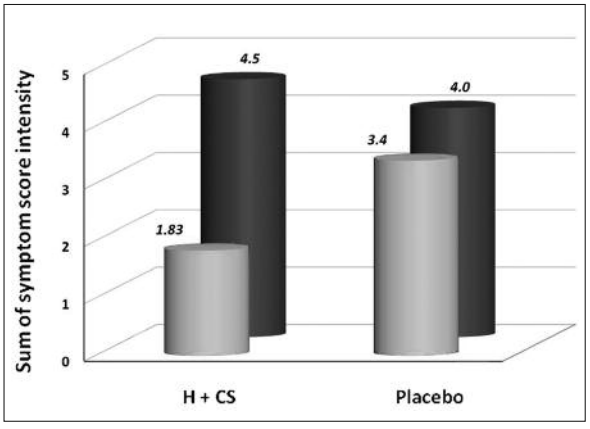

Figure 1. Sum of Symptoms Score Intensity absolute values after randomized sequences completion (*$p < 0.01$ between group). H+CS = hyaluronic acid and chondroitin-sulphate. Black bars indicate sum of symptom score intensity before treatment; Gray bars indicate sum of symptom score intensity after treatment.

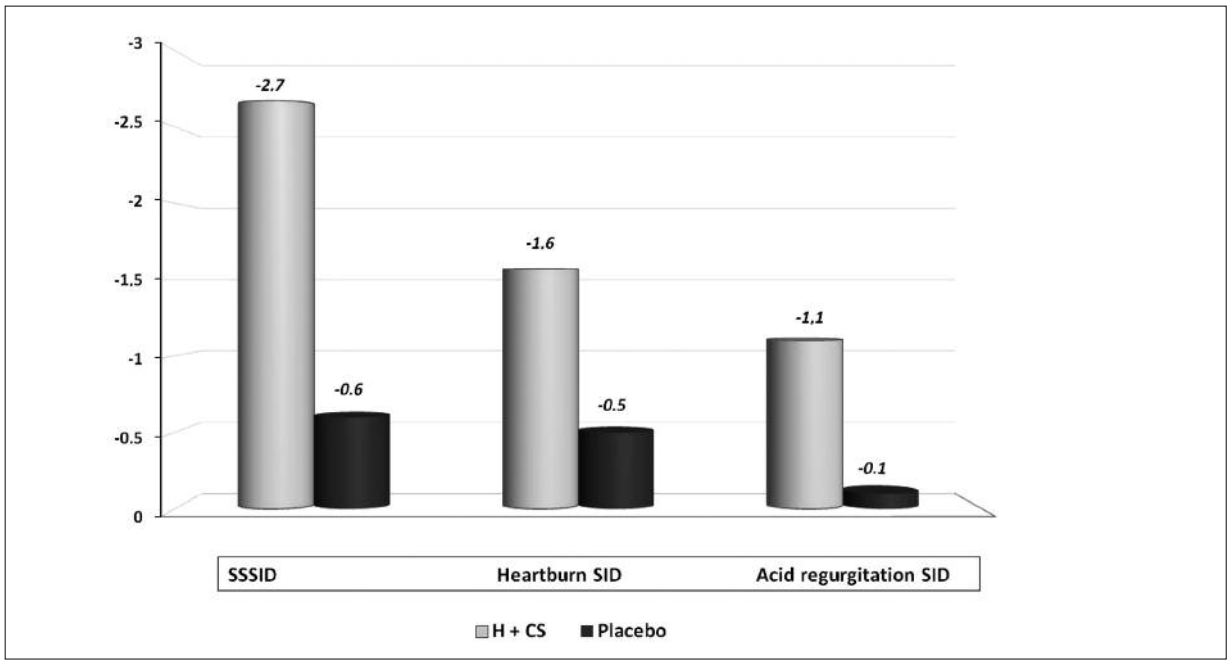

Figure 2. Symptoms Score Intensity Difference (SSID) and heartburn and acid regurgitation score intensity difference after randomized sequences completion. H+CS = hyaluronic acid + chondroitin-sulphate; *$p < 0.03$ (heartburn SID, acid regurgitation SID); **$p < 0.01$ (SSID).

0.01, Figure 2) as result of significant changes in heartburn intensity (-1.6 ± 0.92 vs- 0.5 ± 1.9, $p < 0.03$) and acid regurgitation intensity (–1.1 ± 0.6 vs –0.1 ± 1.1 $p < 0.04$), after HA+CS administration. SSIDs in each of the treatment phase with weekly mean values, deriving from patient's diary are summarized in Figure 3. From the first week of treatment onward, the SSID values were always higher compared to placebo and maximal after the second week of treatment whatever the randomized sequence.

Symptoms complete disappearance was higher after HA+CS treatment: 52% vs 12% ($p = 0.01$ – Figure 4). The time to disappearance of symptoms in the HA+CS group was significantly shorter than placebo (median 38 min vs 65 min – $p < 0.01$) and HA+CS treatment exhibited an higher, statistically significant, percentage of patients reporting good speed of action (≤ 30 min) compared to placebo (60% vs 30% respectively – $p = 0.05$, Table II).

Rapid onset of action was particularly observed when HA+CS syrup was administered as first drug in the sequence (70% of patients with a speed of action ≤30 min) Beneficial effects lasted for more than 3 hours in 60% of patients during therapy with HA+CS compared to only 25% during placebo treatment.

Percentage of patients using antacids and antacids weekly assumption did not change during both treatments administration.

HA+CS syrup taste was considered as pleasant by 80% of patients.

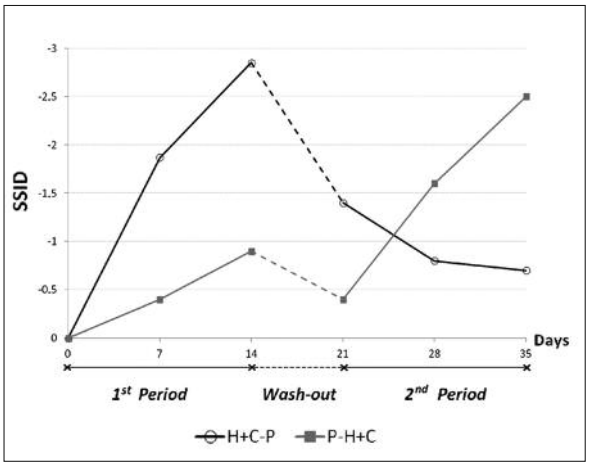

Figure 3. Sum of Symptoms Score Intensity Difference weekly values at each time point. H+CS = hyaluronic acid and chondroitin-sulphate; P = placebo; SSID = Symptoms Score Intensity Difference. Sequence hyaluronic acid + chondroitin-sulphate-placebo values are represented in black lines, sequence placebo-hyaluronic acid + chondroitin-sulphate in blue lines. Wash out period values are indicated with dashed lines.

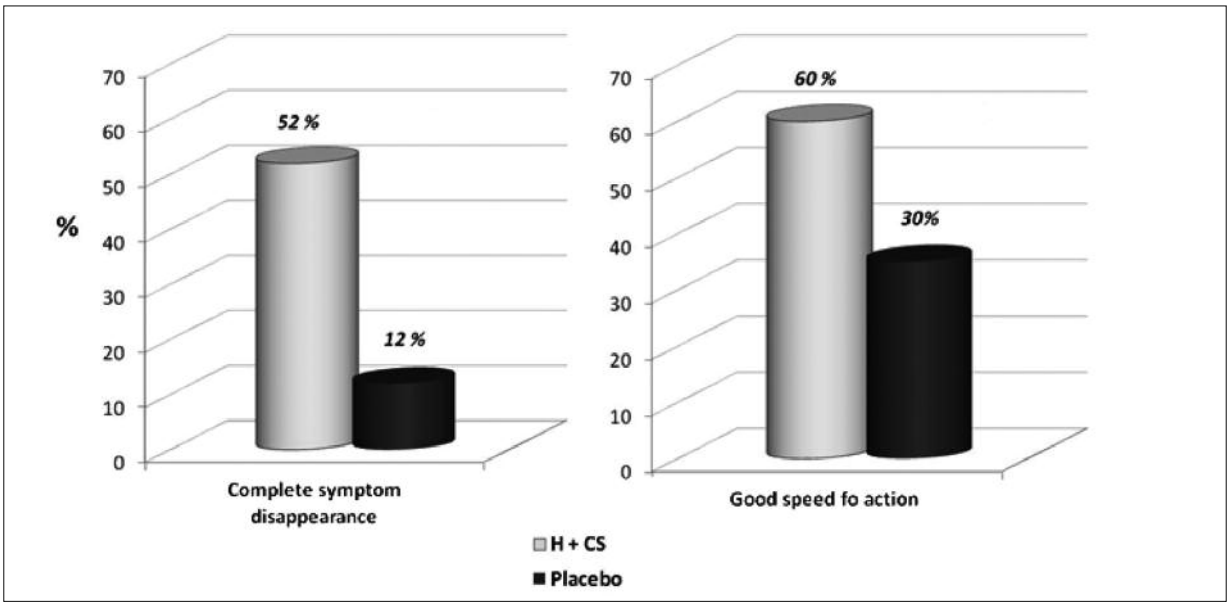

Figure 4. Rate of patients with complete symptom disappearance and rate of patients reporting good speed of action (≤ 30 min). H+CS = hyaluronic acid and chondroitin-sulphate; $p = 0.01$ (complete symptom disappearance); $p = 0.05$ (*good speed*).

A total of 9 AEs, mainly consisting of gastrointestinal complaints (diarrhea, abnormal bowel habit, gastrointestinal discomfort, nausea) were reported by 7 patients: 4 AEs in 3 patients during the HA+CS administration and 5 AEs in 4 patients during placebo treatment.

Discussion

Our study proves the efficacy of adding an oral formulation of hyaluronic acid and chondroitin sulphate to a PPI for the treatment of patients with NERD, that could have a lower response rate to PPIs than patients with erosive esophagitis, especially when evaluated on heartburn relief[10]. NERD etiology includes altered esophageal motility, visceral hypersensitivity, impaired esophageal mucosal barrier function. The HA+CS effect should be looked at with interest given that the increased permeability due to chemical damage induced by pathological gastroesophageal reflux represents the main mechanism for the development of the mucosal breaks and symptoms, such as pain independently from detectable lesions[20].

The esophagus is a food and fluids transit GI segment, with very short transit time hampering local drug delivery and prolonged chemical tissue interaction. Our new medical device, in its formulation background has been conceived with the rationale of a mucosa coating-lubricating-hydrating action mechanism; in fact, usually, the esophageal mucosa is protected against mechanical and chemical injuries by stratified multilayered squamous epithelium which represents a true mucosal barrier. Physiologically, the salivary flow enhances the mucosal defences, but when some environmental imbalance is generated, our regularly administered active principles can ac-

Table II. Speed of action, distribution by treatment.

Speed of action	Hyaluronic acid + Condroitin-sulphate No (%)	Placebo No (%)	*p* value
≤ 15 min	2 (10)	1 (5)	
> 15 min and ≤ 30 min	10 (50)	5 (25)	
> 30 min (max 90 min)	8 (40)		
	14 (70)		
Total of ≤ 30 min	12 (60)	6 (30)	0.05

tively replace the mucosal protection and promote repair; in fact, all the damaging chemicals such as foods, hydrochloric acid and pepsin contained in the gastric refluxing fluid may impair the barrier and subsequently, increase the mucosal permeability[20] as in symptomatic GERD or gastritis affected patients.

HA is an high molar mass glycosaminoglycan, able to organize a reticular structure and a molecular framework as a filter to prevent the diffusion of high-molecular weight substances[16] exploiting its viscoelastic properties linked to its polymeric and hydrating features. The putative HA mechanism of action strongly supports the induction of epithelial cells shifting. Increased motility at adequate HA concentration to cover the submucosal connective tissue which become soft and hydrophilic beneath the fibrin to repair damaged mucosal layer. CS may be of benefit in diseases where inflammation is an essential marker[18]. The efficacy of chondroitin sulphate should be due not only to the affinity between its sulphonated molecular structure and the aminic groups of pepsin molecule, but also to the induction of a wide range of proteinated complexes coating steadily and protecting the deepithelized or ulcerated esophageal-gastroduodenal areas. In addition, the ability of the bioadhesive polymer to produce a persistent mucosal barrier effect was also demonstrated. For this reason we tested the hypothesis that prevention of the increase permeability due to the presence of mucosal breaks could be accomplished with HA+CS which can coat the damaged mucosa with its component. Ulcer and erosion healing effect of CS is thus synergistically co-promoted by HA and the adhesive biopolymer (added to prolong the coating-healing action of the two mixed natural compounds). On the other hand, in the swine experimental model of esophageal mucosa damage, like in the human gastroesophageal reflux, Di Simone was able to reduce the permeability of the esophageal injured mucosa, with the identical formula adopted in a nutraceutical product (Esoxx, Alfa Wassermann Spa, Bologna, Italy).

Conclusions

The results of this study show that treatment with a fixed combination of hyaluronic acid and chondroitin-sulphate produced a fast relief of GERD symptoms. Secondary analysis showed that significantly more patients receiving HA+CS achieved rapid symptoms disappearance and prolonged symptoms free period. These characteristics make HA+CS a valid tool for treatment of GERD symptoms in NERD patients.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Dent J, El-Serag HB, Wallander MA, Johansson S. Epidemiology of gastroesophageal reflux disease: a systematic review. Gut 2005; 54: 710-717.
2) Vakil N, van Zanten SV, Kahrilas PJ, Dent J, Jones R. Global Consensus Group. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. Am J Gastroenterol 2006; 101: 1900-1920.
3) Kahrilas PJ. Clinical practice. Gastroesophageal reflux disease. N Engl J Med 2008; 339: 1700-1707.
4) Galmiche JP, Bruley des Varannes S. Symptoms and disease severity in gastro-oesophageal reflux disease. Scand J Gastroenterol 1994; 201: 62-68.
5) Richter JE. The many manifestations of gastroesophageal reflux disease: presentation, evaluation and treatment. Gastroenterol Clin North Am 2007; 36: 577-599.
6) Modlin IM, Hunt RH, Malferheiner P, Moayyre P, Quigley EM, Tytgat GN, Tack J, Heading RC, Holtman G, Moss SF. Vevey NERD Consensus Group. Diagnosis and management of non-erosive reflux disease. Digestion 2009; 80: 74-88.
7) Mainie I, Tutuian R, Shay S, Vela M, Zhang X, Sifrim D, Castell DO. Acid and non-acid reflux in patients with persistent symptoms despite acid suppressive therapy. A multicentre study using combined ambulatory impedance-Ph monitoring. Gut 2006; 55: 1398-1402.
8) Sifrim D, Castell D, Dent J, Kahrilas PJ. Gastroesophageal reflux monitoring, review and consensus report on detection and definitions of acid, non acid and gas reflux. Gut 2004; 53: 1024-1031.
9) Tack J. Review article: the role of bile and pepsine in the pathophysioloigy and treatment of gastroesophageal reflux disease. Aliment Pharmacol Ther 2006; 24(Suppl 2): 10-16.
10) Dean BB, Gano AD, Knight K, Ofman JJ, Fass R. Effectiveness of proton pump inhibitors in non erosive reflux disease. Clin Gastroenterol Hepatol 2004; 2: 656-664.
11) Galmiche JP, Stephenson K. Treatment of gastroesophageal reflux disease in adults: an individual approach. Dig Dis 2004; 22: 148-160.

12) Weiberg DS, Kadish SJ. The diagnosis and management of gastro-oesophageal reflux disease. Med Clin N Amer 1996; 80: 411-429.

13) Wang C, Hunt RH. Medical management of gastroesophageal efflux disease. Gastroenterol Clin North Am 2008; 37: 879-899.

14) Fass R, Sifrin D. Management of heartburn not responding to proton pump inhibitors. Gut 2009; 58: 295-309.

15) Fass R, Shapiro M, Dekel R. Systematic review: proton-pump inhibitor failure in gastro-oesophageal reflux disease, where next? Aliment Pharmacol Ther 2005; 25: 79-94.

16) Volpi N, Schiller J, Stern P, Soltes I. Role, metabolism, chemical modification and application of hyaluronan. Curr Med Chem 2009; 16: 1718-1745.

17) Gaffney J, Matou Nasri S, Grau-Olivares M, Stevin M. Therapeutic applications of hyaluronan. Mol Byosyst 2010; 6: 437-443.

18) Lauder RM. Chondroitin sulphate a complex molecule with potential impacts on a wide range of biological systems. Complement Ther Med 2009; 17: 56-62.

19) Du Souich P, Garcia AG, Verges J, Montell E. Immunoimodulatory and anti-inflammatory effects of chondroitin sulphate. J Cell Mol Med 2009; 13: 1451-1463.

20) Batchelor HK, Tang M, Dettmar PW, Hampson FC, Jolliffe IG, Craig DQ. Feasibility of a bioadhesive drug delivery system targeted to oesophageal tissue. Eur J Pharm Biopharm 2004; 57: 295-298.

21) Orlando RC. The integrity of the esophageal mucosa. Balance between offensive and defensive mechanisms. Best Res Clin Gastroenterol 2010; 24: 873-882.

22) Orlando LA, Orlando RC. Dilated intercellular spaces as a marker of GERD. Curr Gastroenterol Rep 2009; 11: 190-194.

23) Vakil N. Endoscopy in GERD: boondoggle. Diagnostic test or risk management tool? Am J Gastroenterol 2008; 103: 276-278.

24) Epple HJ, Kirstein FW, Bojarski C, Frege J, Fromm M, Riecken EO, Schulzke JD. 13C-urea breath test in Helicobacter Pylori diagnosis and eradication correlation to histology,origin of false results and influence of food intake. Scand J Gastroenterol 1997; 32: 308-314.

25) Carlsson R, Dent J, Bolling-Sterevald E, Johnsson F, Junghard O, Lauritsen K, Riley S, Lundell L. The usefulness of a structured questionnaire in the assessment of symptomatic gastroesophageal reflux disease. Scand J Gastroenterol 1998; 33: 1023-1029.

26) Chevrel B. A comparative crossover study on the treatment of heartburn and epigastric pain: liquid Gaviscon and a magnesium-aluminium antacid gel. J Int Med Res 1980; 8: 300.