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Savarino V, et al. Submitted for publication.
Barrier effect of ESOXX® on esophageal mucosal damage: experimental study on ex-vivo swine model


Introduction

• ESOXX® ONE is a medical device for the topical treatment of the symptoms of GastroEsophageal Reflux Disease (GERD).

• The product is based on a mixture of Hyaluronic Acid (HA) and Chondroitin Sulphate (CS) in a bioadhesive carrier (poloxamer 407), which acts as a buffering agent to form a barrier prolonging the action on esophageal mucosa[1].

• HA is a glycosaminoglycan that is mainly present in the extracellular matrix of soft connective tissues and involved in several key processes, including cell signalling, wound repair and regeneration, morphogenesis, matrix organisation and pathobiology[2].

• CS is a natural glycosaminoglycan present in the extracellular matrix and is formed by the 1-3 linkage of D-glucuronic acid to N-acetylgalactosamine, and may be of benefit in diseases where inflammation is an essential marker[3,4].

Study design

• This study evaluated the barrier effect of ESOXX® ONE on the esophageal mucosa using an ex-vivo swine esophagus model.

Materials and methods

• Mucosal damage to swine esophagus was induced by 15 to 90 min of perfusion with an acid solution (HCl, pH 1.47) with or without pepsin (2000 U/ml, acidified to pH 2), and 30 esophageal specimens were histologically evaluated; Evans
Blue (EB) dye solution was used to assess the permeability of swine mucosa after chemical injury.

- The severity of the lesions induced by damaging solutions on the swine esophageal mucosa is shown in Table 1. Histologic damage was found to be directly related to perfusion time. The acid solution alone did not show evident histological damage, when perfused for 15 minutes, while more prolonged perfusion caused a progressive involvement of the inner mucosal layers.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Acid solution</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>9 (100%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>30</td>
<td>1 (11%)</td>
<td>7 (78%)</td>
<td>1 (11%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>60</td>
<td>–</td>
<td>1 (11%)</td>
<td>7 (78%)</td>
<td>1 (11%)</td>
<td>–</td>
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<tr>
<td>90</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>9 (100%)</td>
<td>–</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Pepsin solution</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>–</td>
<td>7 (78%)</td>
<td>2 (22%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>30</td>
<td>–</td>
<td>–</td>
<td>7 (78%)</td>
<td>2 (22%)</td>
<td>–</td>
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<tr>
<td>60</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>9 (100%)</td>
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</tr>
</tbody>
</table>

Notes: Data expressed as absolute frequency (%) of score attributed (grade 0 = no damage; grade 1 = mild damage; grade 2 = moderate damage; grade 3 = severe damage).

Table 1
Severity of histological damage induced with acidic and pepsin solutions.

- Adding pepsin to the acid solution, during the damage induction phase, produced histological damage at earlier time points. Severe histological lesions were always observed after acid and pepsin perfusion for 90 and 60 min, respectively.

- In order to assess the permeability of damaged esophageal mucosa, the esophagi were divided in three portions. The first (used as positive control) was perfused with Evans Blue (EB) dye solution (10 minutes at 1 ml/minute); the second, with ESOXX® ONE (10 minutes, 1 ml/minute) followed by EB dye
solution (10 minutes at 1 ml/minute); the third segment was perfused with ESOXX® ONE (10 minutes at 1 ml/minute), then washed with saline solutions for 30 seconds, and finally perfused with EB dye solution (10 minutes at 1 ml/minute). The EB staining of the portions was measured to show the permeability to EB dye of the mucosa and was scored with a 3-point scale: 0 (no stain); 1 (weak stain); 2 (strong stain). Two undamaged esophagi as the negative control and six esophagi with severe mucosal lesions were also used.

**Results**

- No staining was detected on the epithelial layer of control mucosa and ESOXX® ONE, while EB staining was evident in all the samples of damaged mucosa with 67% of them showing a strong stain (Table 2).
- The perfusion of ESOXX® ONE after damage with acid solution for 90 min and pepsin solution for 60 min was able to completely prevent the EB staining in all the mucosal samples examined (Figure 1).
- This effect of ESOXX® One was not reversed by a short period of saline perfusion (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>0 (no stain)</th>
<th>1 (weak stain)</th>
<th>2 (strong stain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No damaged mucosa + EB</td>
<td>2 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Damaged mucosa + EB</td>
<td></td>
<td>2 (33%)</td>
<td>4 (67%)</td>
</tr>
<tr>
<td>Damaged mucosa + ESOXX® ONE + EB</td>
<td>6 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Damaged mucosa + ESOXX® ONE + saline + EB</td>
<td>6 (100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Data expressed as frequency of staining score observed in each experimental group (six mucosal samples).

**Table 2**
Evans Blue (EB) staining on control tissue and damaged mucosa with or without ESOXX® ONE application.
Conclusions

- Mucosal damage is associated with an increased permeability, documented by evident EB staining.
- Perfusion with ESOXX® ONE is able to reduce the permeability of the injured mucosa, even after saline washing of the swine esophagus.
- The authors concluded that these preliminary results support further clinical studies of ESOXX® ONE in the treatment of GERD symptoms.

References

Preliminary clinical experience with a new natural compound in the treatment of esophagitis and gastritis: symptomatic effect


Introduction

• Symptomatic treatment of esophagitis and gastritis is primarily approached with Proton-Pump Inhibitors (PPIs) to reduce acid output together with buffering products that counteract hydrogenionic damage to the mucosa.

• These authors undertook an original approach, identifying compounds that could buffer the acidity of gastric fluid and the pepsin-induced mucosal rebound damage by coating the epithelial surface as long as possible to stimulate the healing process.

• Hyaluronic Acid (HA) and Chondroitin-Sulphate (CS) have been previously identified as candidate molecules that may promote healing of esophageal lesions while providing a barrier effect[1,2].

Study design

• This preliminary clinical, placebo-controlled double-blind crossover study was carried out on 40 patients with esophageal and gastric symptoms, 10 of whom were affected by reflux disease.

Materials and methods

• The study enrolled 16 females and 24 males aged between 6 and 87 years (average 55-85), with esophageal and gastritic symptoms characterised by heartburn, epigastric pain, dyspepsia, meteorism and belching.
• **ESOXX®,** (syrup) or placebo were administered with the following schedule: one spoon (about 10 ml) every 8 hours (far from meals) and two spoons at bedtime for two weeks.

• This was followed by a one-week interval without administration, and two weeks of treatment were then carried out by switching placebo and active principle in the groups; symptoms were then scored.

**Results**

• In the group starting on **ESOXX®** there was significant improvement of symptoms score vs. placebo.

• Less benefit was seen in patients starting on placebo before switching to **ESOXX®,** which was attributed to the extension of the period with symptoms before the treatment with **ESOXX®**.

• Pre-post treatment endoscopic investigations showed improvement of inflammation and healing of the mucosa in patients with either esophageal or gastroduodenal pathologies (**Figure 1**).

![Figure 1](image)

**Figure 1**
Pre-post treatment endoscopic investigations.

(A) Mucosal erosions of the esophagus;
(B) Mucosal erosions of the esophagus after therapy;
(C) pyloric ulcer;
(D) absence of pyloric ulcer after therapy.

• The efficacy was meaningful, especially in children with reflux and adults with
biliary gastritis probably due to prompt neutralisation of alkaline biliary fluid.

- Twelve patients with esophagitis had total remission of symptoms; 7 had <60% symptom reduction and 1 <30% symptom reduction.
- There were no adverse events during the trial.

**Conclusions**

- Statistically significant differences in symptom relief were seen with ESOXX® vs. placebo.
- Pre-post treatment endoscopic investigations also showed improvement of inflammation and healing of the mucosa in esophageal or gastroduodenal pathologies.
- Overall, ESOXX® was considered useful in controlling esophageal and gastric symptoms of reflux and inflammation.

**References**


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


Introduction

• Current medical management of GastroEsophageal Reflux Disease (GERD) is based on the administration of acid secretion inhibitors such as Proton-Pump Inhibitors (PPIs)[1-4].

• In more than 30% of patients, PPI therapy fails to completely resolve symptoms. This number is even higher in Non-Erosive Reflux Disease (NERD) patients where failure rates of >40% have been reported[5,6].

• A new medical device (ESOXX® ONE) based on a combination of Hyaluronic Acid and Chondroitin-Sulphate (HA+CS) in a bioadhesive carrier (Poloxamer 407) may constitute a modern approach to relief the symptoms of GERD.

• HA, mainly present in the extracellular matrix of soft connective tissues, is involved in several key processes such as control of epithelial cell turnover, favouring re-epithelisation and mucosal hydration in ulcer healing[7].

• CS is a safe glycosaminoglycan that is able to inhibit pepsin induced damage of the gastroduodenal mucosa, and may be of benefit in disease where inflammation is an essential marker[8,9].

• The bioadhesive carrier (Poloxamer 407) 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[10].
Study design

• The aim of this study was to evaluate the effect of a new medical device, based on an oral fixed combination of HA+CS in a bioadhesive carrier in adults with symptoms of NERD and with a low response to PPIs.

• This was a randomised double-blind, crossover, placebo-controlled study in 20 patients (17 males, 3 females; mean age 55±18; mean BMI 28.3±5) who had experienced heartburn and/or acid regurgitation for at least 3 days during a 7 day run-in period, without endoscopic mucosal breaks.

Materials and methods

• Patients, under PPI therapy, received four daily doses of ESOXX® ONE or placebo for a period of 14 days. After a washout period of 7 days, patients were then crossed over to receive ESOXX® ONE or placebo for an additional 14 days.

• PPI at standard dose were maintained over all the study period.

• Intensity of heartburn and acid regurgitation at awakening and/or at bedtime, were recorded daily by 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).

• 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.

Results

• SSSI absolute value at the end of HA+CS treatment was significantly lower compared to placebo treatment (from 4.5±1.4 to 1.8±2.2 and from 4.0±2.1 to 3.4±1.9 respectively, p<0.01), whatever was the randomised sequence (Figure 1).
A statistically significant SSID was observed (-2.7±1.4 vs. -0.6±2.1, p<0.01) 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 ESOXX® ONE administration (Figure 2).

Figure 1
Sum of Symptoms Score Intensity (SSSI) absolute values after randomised sequences completion. H+CS: Hyaluronic Acid + Chondroitin Sulphate.

Figure 2
Symptoms Score Intensity Difference (SSID), heartburn and acid regurgitation Score Intensity Difference (SID) after completion of randomised sequences. HA+CS: Hyaluronic Acid + Chondroitin Sulphate. Adapted from Palmieri et al. 2013.
• SSIDs in each of the treatment phase with weekly mean values, deriving from patient’s diaries are summarised in Figure 3. From the first week of treatment onward, SSID values were always higher compared to placebo and maximal after the second week of treatment whatever the randomised sequence.

![Figure 3](image1.png)

**Figure 3**
Sum of Symptoms Score Intensity Difference weekly values at each time point.
HA+CS: Hyaluronic Acid+Chondroitin Sulphate; SSID: Symptoms Score Intensity Difference.

• Complete disappearance of symptoms was higher after ESOXX® ONE treatment: 52% vs. 12% of placebo (p=0.01) (Figure 4).

![Figure 4](image2.png)

**Figure 4**
Percentage patients with complete symptom disappearance and reporting good speed of action (≤30 min). HA+CS: Hyaluronic Acid+Chondroitin Sulphate.
• The time to disappearance of symptoms in the ESOXX® ONE group was significantly shorter than placebo (median 38 min vs. 65 min; p<0.01) and ESOXX® ONE treatment was associated with a higher, statistically significant percentage of patients reporting good speed of action (≤30 min) compared to placebo (60% vs. 30% respectively; p=0.05) (Figure 4).

• The beneficial effects lasted for more than 3 hours in 60% of patients during therapy with ESOXX® ONE compared to only 25% during placebo treatment.

• A total of 9 Adverse Events (AEs), mainly consisting of gastrointestinal complaints (diarrhoea, abnormal bowel habit, gastrointestinal discomfort, nausea) were reported by 7 patients: 4 AEs in 3 patients during ESOXX® ONE administration and 5 AEs in 4 patients during placebo treatment.

Conclusions

• The study concluded that treatment with ESOXX® ONE produced a fast relief of GERD symptoms.

• Secondary analyses showed that significantly more patients receiving ESOXX® ONE achieved rapid disappearance of symptoms and a prolonged symptom-free period.

• Taken together, ESOXX® ONE was considered to be a valid tool for treatment of GERD symptoms in NERD patients.
References


Efficacy and safety of a 15-day treatment with an oral combination of hyaluronic acid and chondroitin-sulphate as add-on therapy to a proton pump inhibitor in patients with NERD

Savarino V, et al. Submitted for publication.

Introduction

• Gastroesophageal reflux can represent a pathological condition called GastroEsophageal Reflux Disease (GERD) when it causes symptoms or complications associated with significant morbidity. Symptoms such as heartburn and acid regurgitation occur weekly in about 20% of the adult population[1].

• 60% of patients with typical GERD symptoms do not present evidence of mucosal damage (Non-Erosive Reflux Disease, NERD)[2].

• ESOXX® ONE, a new medical device for the treatment of the symptoms of GERD, is based on a synergistic mixture of Hyaluronic Acid (HA), Chondroitin-Sulphate (CS) and a bioadhesive carrier (Poloxamer 407)[2,3].

• ESOXX® ONE acts as a buffering agent to form a barrier and prolong the action on esophageal mucosa[2,3].

• For its unique characteristics, ESOXX® ONE has been studied as a protective topical agent towards esophageal and gastric lesions[2,3].

Study design

• The aim of this multicentre, randomised, double-blind, placebo-controlled study was to evaluate the efficacy and safety of 15-day treatment with an oral combination of HA and CS (ESOXX® ONE) as add-on therapy to a Proton-Pump Inhibitor (PPI) in patients with NERD.
Materials and methods

- Patients must have had a diagnosis of NERD and at least 2 of the typical symptoms of GERD, namely heartburn, retrosternal pain, regurgitation or acid taste in the mouth, from at least 3 months and at least 3 days per week in the months preceding study screening visit.

- Following the screening visit, 154 NERD patients under a PPI therapy, underwent a 15-day washout period without the PPI administration. At the randomisation visit, 76 patients were assigned to ESOXX® ONE and 78 to placebo for 15 days in addition to PPI at standard dose.

- The RDQ questionnaire was used to evaluate symptoms: frequency and severity (on a 4 point Likert scale) of symptoms: heartburn, retrosternal pain, acid regurgitation and acid taste in the mouth, to obtain the total GERD symptoms score.

- The primary endpoint was the percentage of patients with at least 3 points reduction in the GERD total symptoms score collected through the patient’s RDQ at final visit.

Results

- A significantly higher percentage of patients with reduction of total symptom score ≥3 points were seen in patients treated with ESOXX® ONE vs. placebo in the Intention To-Treat (ITT) population (primary endpoint) (Figure 1).

- Considering the percentage of patients with a 50% reduction in total symptoms score at final visit, significant differences vs. placebo were seen in the ITT population (Figure 2).

- Significant differences between ESOXX® ONE and placebo were seen considering the percentage of patients with any reduction in total symptoms score at final visit (78.9% vs. 56.4%; p=0.003; ITT population).

- The average reduction of total symptoms score with ESOXX® ONE at final visit was double than that seen in the placebo group (-3.1±3.1 vs. -1.5±3.0, p=0.002; ITT population).
There was also a significant reduction in the number of days with symptoms with ESOXX® ONE vs. placebo (-6.07 vs. -3.96; p=0.0059; ITT population).

The patients’ quality of life, evaluated by a SF-36 questionnaire, showed statistically significant differences, in favor of ESOXX® ONE group, for general health perception (difference between the adjusted means of the ESOXX® ONE and the placebo group: -4.8006; 95% CI: -8.4409 to -1.1603; p=0.0101) and social function (difference between the adjusted means of the ESOXX® ONE and the placebo group: -5.2620; 95% CI: -9.7049 to -0.8190; p=0.0206; ITT population).

Figure 1
Percentage of patients with a reduction in total symptoms score of at least 3 points at final visit in the ITT population.

Figure 2
Percentage of patients with 50% reduction in total symptoms score at final visit in the ITT population.
• Considering the palatability of ESOXX® ONE, 90% of patients reported that administration was acceptable (excellent, good, indifferent), independently from meals or bedtime, vs. 92% with placebo (ITT population).

Conclusions
• In this study, ESOXX® ONE was found to be able to double the efficacy of the treatment, when combined to PPI, in NERD patients.
• It was also considered to have a good safety profile, was well-tolerated and highly palatable.

References
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Nonerosive reflux disease—current concepts and dilemmas. 

2 Palmieri B, Merighi A, Corbascio D, Rottigni V, Fistetto G, Esposito A. 
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Barrier effect of esoxx((r)) on esophageal mucosal damage: Experimental study on ex-vivo swine model. 