Thesis title:

Lower Esophageal Sphincter Augmentation by Endoscopic Injection of Dextranomer Hyaluronic Acid Copolymer: Preliminary Findings in A Porcine Gastroesophageal Reflux Disease Model

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Submitted on: August 2013

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Masters of Experimental Surgery (MSc.)

Contents

Abstract

Background:

We previously demonstrated feasibility, safety, and a reproducible histologic bulking effect after injection of dextranomer hyaluronic acid copolymer (DxHA) into the gastroesophageal junction (GEJ) of rabbits. In the current study, we investigated the potential for DxHA to augment the lower esophageal sphincter (LES) in a porcine model of gastroesophageal reflux disease (GERD).

Methods:

12 Yucatan miniature pigs underwent LES manometry and 24-hour ambulatory pH monitoring at baseline, after cardiomyectomy, and 6 weeks after randomization to endoscopic injection of either DxHA or saline at the LES. After necropsy, the foregut, including injection sites, was histologically examined.

Results:

Pigs in both groups had similar weight progression during the study period, with two mortalities prior to randomization. Cardiomyectomy successfully induced GERD in all animals measured by a rise in the median percentage of time pH<5 from 0.6% to 11.6% (p=0.02). Endoscopic injection of DxHA resulted in a higher median difference in LES length (1.8 cm vs. 0.4 cm, p=0.03), 120% increase in the median LES pressure difference (median change of pressure -0.5 mmHg in saline group, 0.1 mmHg in DxHA group, p=0.8), and 124% decrease in the median difference in the number of reflux episodes in DxHA group in comparison with saline group (p=0.6). Histologically, injection of DxHA induced a foreign body reaction with fibroblasts and giant cells.

Conclusions:

Porcine cardiomyectomy is a reproducible animal GERD model. Injection of DxHA augments the LES. Further studies are required to elucidate a potential therapeutic effect in human GERD.

Résumé

Contexte:

Nous avons auparavant démontré la faisabilité, l'absence de danger et un effet de grossissement histologique reproductible après l'injection de copolymère d'acide hyaluronique/dextranomère (DxHA) dans la jonction gastroesophagienne (GEJ) de lapins. Dans notre étude, nous avons étudié la capacité du DxHA d'augmenter le sphincter inférieur de l'œsophage (SIO) dans un modèle porcin de la maladie de reflux gastroesophagien (GERD).

Méthodes:

Douze porcs miniatures du Yucatan ont été soumis à une manométrie du SIE et à une surveillance du pH ambulatoire de 24 heures au niveau de base, après une cardiomyectomie, et de six semaines après des injections aléatoires de DxHA ou de solution saline au SIO. Après l'autopsie, l'intestin antérieur, y compris les points d'injection, ont été examinés histologiquement.

Résultats:

Les poids des porcs des deux groupes ont progressé de façon semblable durant la période de l'étude, et il y a eu deux mortalités avant les injections aléatoires. La cardiomyectomie a induit avec succès une maladie de reflux gastroesophagien (GERD) chez tous les animaux, qui a été mesurée par une augmentation du pourcentage médian du pH temps < 5 de 0,6 % à 11,6 % (p = 0,02). L'injection endoscopique de DxHA a provoqué une différence médiane plus élevée dans la longueur du SIO (1,8 cm plutôt que 0,4 cm, p = 0,03), une augmentation de 120 % dans la différence de pression médiane dans le SIO (variation médiane de pression de -0,5 mm de Hg dans le groupe ayant reçu

des injections salines et de 0,1 mm de Hg dans le groupe ayant reçu des injections de DxHA, p = 0,8) et une diminution de 124 % dans la différence médiane du nombre de périodes de reflux du groupe ayant reçu du DxHA comparativement à celui ayant reçu une solution saline (p = 0,6). Histologiquement, l'injection de DxHA a produit une réaction à un corps étranger qui s'est manifestée par des fibroblastes et des cellules géantes.

Conclusions:

La cardiomyectomie porcine est un modèle animal reproductible de la maladie de reflux gastroesophagien (GERD). L'injection de DxHA augmente le SIO. D'autres études sont nécessaires pour élucider un effet thérapeutique éventuel applicable à la GERD humaine.

Acknowledgments

First and foremost I offer my sincerest gratitude to my supervisor, Dr. Sherif Emil, who has supported me through my thesis with his patience and knowledge while allowing me the room to innovate and think independently. I attribute the completion of my Master degree to his continuous encouragement and effort. One simply could not wish for a better supervisor.

The good advice and support of my second supervisor, Dr. Jean-Martin Laberge, has been invaluable, for which I am extremely grateful. I also offer special thanks to the rest of my thesis committee members, Dr. John Mort and Dr. Robert Baird for their support, guidance and helpful suggestions.

In my daily work, I have been blessed with a friendly and cheerful assistant, Dr. Sherif Elkady, who helped me execute all the laboratory experiments. I would also like to thank Dr. Mayrand and Dr. Morinvile for teaching me how to perform manometry and pH study, Dr. Blumenkrantz and Dr. Nguyen for educating me about histopathology. I also appreciate the feedback on the statistical analysis by Dr. Tan.

Members of the Montreal General Hospital Large Animal Facility at McGill University also deserve my sincerest thanks especially Mrs. Robitaille and Ms. Browning for taking care of the animals and being very flexible to accommodate a busy experiment schedule. I would also like to express my gratitude to the Saudi Arabian Cultural Bureau for their financial support. Oceana Therapeutics, the previous manufacturer of Deflux®, donated the DxHA syringes for injection.

Above all, I owe my deepest gratitude to my wife Khloud for her love, support and great patience at all times, for which my mere expression of thanks does not suffice.

Contribution of Authors

S.E. primarily supervised the study. S.E., J-M.L. and A.A. jointly designed the study. A.A. designed the scope and the needles with the engineer. S.M. and V.M. supervised manometry and pH measurements. S.E., J-M.L., A.A. and S.EL. performed the surgeries. A.A. and S.EL. performed manometry, pH measurements and necropsy. M.B. and V.N. performed histological examinations. A.A recorded the data, conducted statistical analysis, interpret the results, and wrote the manuscript. S.E. and J-M.L. revised the manuscript.

1.0 Background:

Gastroesophageal reflux disease (GERD) is a very common condition affecting up to 20% of the pediatric population with a higher prevalence in those with neuromuscular disorders [1,2]. In several large population-based surveys, 17-40% of adults reported GERD symptoms within the last three months [3,4]. Although GERD-related mortality is very low, quality of life is significantly impaired in patients with GERD, demonstrating more impairment than patients with diabetes, hypertension, arthritis, or mild heart failure [5-12]. Proton pump inhibitors (PPIs) are the mainstay of medical treatment of GERD in both children and adults [13-15]. PPIs result in 70-80% resolution of symptoms and healing of esophagitis, but to 80% recurrence rate is observed when treatment is discontinued [15-17]. Despite the efficacy of PPIs in adult, it remains suboptimal in reducing GERD-related symptoms in the pediatric population [18-23]. Moreover, safety of PPIs has been questioned in recent reports [19,24-27]. Anti-reflux surgery (ARS), namely laparoscopic fundoplication, is reserved for patients with poor response to medical treatment or GERD-related complications. Although this procedure is one of the most common pediatric surgical operations performed in the United States, studies have shown a wide range of outcomes, with success rates between 75% and 100% [28,29]. In fact, studies by Lee and colleagues have shown no reduction in GERD-related admission one year after fundoplication, and up to 75% of children continuing anti-reflux medications a year after surgery [21,28,30-36]. Recent randomized controlled trials have not proven the advantages of laparoscopic fundoplication in children, previously published in multiple cases series [37,38]. The American Pediatric Surgical Association has encouraged research into alternative therapies for GERD in children [39]. The

stronger enthusiasm for fundoplication in the adult population has been tempered by published medium and long-term results of randomized trials, which failed to show a significant advantage of laparoscopic fundoplication over PPIs [40,41]. When compared to children, ARS is slightly more effective in adults, however, up to 60% of adult patients continue to require PPIs after surgery to control their symptoms [17,42-45]. Perioperative complications after ARS can be as high as 54%, with up to 33% incidence of postoperative dysphagia in those patients [28,46].

Several alternative endoscopic treatments have been developed over the past decades, but have not gained wide acceptance because of either safety or efficacy concerns [47-56]. Some endoscopically injectable polymers had initially shown promising results. However, substantial concern regarding their safety resulted in their recall [51,52]. To our knowledge, no agents are currently approved for this indication.

Dextranomer hyaluronic acid co-polymer (DxHA), also known as Deflux® (Salix Pharmaceuticals, Raleigh, North Carolina, USA) is a biocompatible polymer composed of dextranomer micropspheres, of 80-250 μm in diameter, suspended in stabilized non-animal hyaluronic acid. DxHA induces a foreign body fibrous tissue reaction at the site of injection. It does not migrate, is non-immunogenic, and is stable for long periods with no reports of adverse long-term effects [57]. Since its introduction into the pediatric urologic practice in the late 1990s, this agent has resulted in a paradigm shift in the treatment of vesicoureteral reflux disease (VUR) [58,59]. This agent has also been used in other conditions such as closure of recurrent tracheoesophageal fistula, umbilical hernia, urinary and fecal incontinence, and for cosmetic purposes [58,60-64].

Our group has been interested in the potential role of DxHA as a treatment for GERD. We have previously demonstrated the feasibility and safety of DxHA injection in the gastroesophageal junction (GEJ) in rabbits. Its injection was able to induce a bulking foreign body reaction without causing esophageal obstruction or perforation [65]. The purpose of this study was to investigate the effect of the endoscopic injection of DxHA in the GEJ in a porcine GERD model.

2.0 Methods

Approval of the research protocol was obtained from McGill University Animal Care Committee following the guidelines imposed by the Canadian Council of Animal Care (protocol 2012-7056). Figure 1 depicts the experimental design of the study.

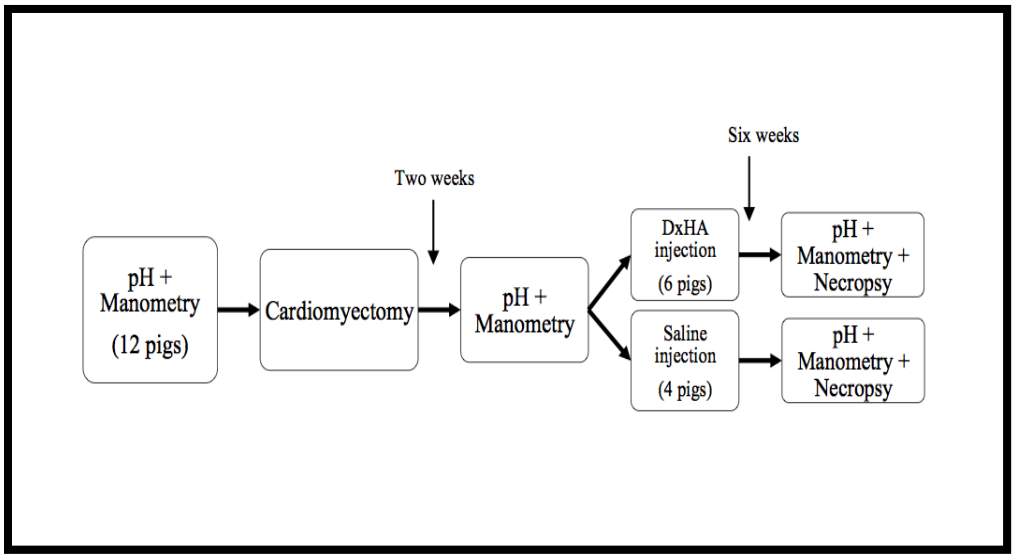

Figure 1: Experimental design.

2.1 Personnel:

The research personnel included two pediatric surgeons, two gastroenterologists, two pathologists, a surgical resident, a research assistant with surgical experience, two animal care technicians, and a veterinarian. All personnel received the necessary animal care training required by the university Animal Care Committee.

2.2 Animals:

Fourteen Yucatan miniature swine weighing 8.4±1.3 kg (obtained from Memorial University, Newfoundland, Canada) were used for the experiment. This strain was specifically chosen because of its slow growth curve, as well as record of success with previous surgical studies. Once received from the vendor, each piglet was placed in a separate cage. All cages were equipped with feeders, and a sipper providing unlimited access to water. The swine were fed a basic research diet Teklad Miniswine® (Harlan Laboratories, Indianapolis, Indiana, USA). Every day, the cage was cleaned and all pigs underwent a clinical examination.

Pigs were started on liquid diet (Ensure®, 1 bottle twice a day) starting 48 hours before each procedure, and allowed water only for 24 hours prior to the procedure. All procedures were performed under general anesthesia and endotracheal intubation. On the day of the procedure, the animals were sedated using intramuscular injection with a mixture of Butorphanol (0.1 mg/kg), Acepromazine (0.2 mg/kg), and Atropine (0.05 mg/kg). After a period of a minimum of 15 minutes, they were given Ketamine intramuscularly (0.14 ml/kg). Anesthesia was induced using Isoflurane inhalant at a 5% delivery or via intravenous Propofol induction (10 mg/ml) titrated to effect. The animals were then intubated in dorsal or lateral recumbent position with a 5.5 mm cuffed

endotracheal tube. Upon achieving the desired effect, an intravenous 22-gauge catheter was introduced into the auricular vein. Lactated Ringers solution was infused at a rate of 10 ml/kg/hour during the procedure. Anesthesia was maintained with Isoflurane at a rate of 2% for the remainder of the procedure.

2.3 Preliminary tests:

Prior to initiating the study, manometry and 24-hour ambulatory pH measurements were performed on two animals to confirm feasibility and ascertain that the tracings obtained can be interpreted by the research team. Subsequently, a laparoscopic cardiomyectomy was attempted on both pigs. However, both procedures required conversion in order to adequately identify the GEJ and all the muscle layers. It became clear that laparoscopy is inferior to the open technique with respect to the ability to identify the esophageal and gastric muscle fibers, the adequacy of cardiomyectomy, and the risk of accidental perforation especially on the gastric side.

2.4 Lower esophageal sphincter manometry:

Esophageal manometry was performed using a commercial stationary water-perfused manometry system including a low-compliance pneumohydraulic capillary infusion pump with a flow rate of 0.5 ml/min (model PIP-4-8, MUI Scientific, Mississauga, Ontario, Canada), a polygraph (Medtronic, Ontario, Canada), and a commercial computer. The four-channel water perfused esophageal catheter (PE 4-3-3-3, MUI Scientific, Mississauga, Ontario, Canada) was used for manometry with four sensors 3 cm apart from each other and with a radial orientation of 90°. This allowed for the collection of four data sets for a given site within the esophagus with a single dynamic measurement. At each level along the esophagus, a mean pressure was obtained

from all the four sensors.

This procedure was done for each pig under general endotracheal anesthesia as described. After priming the manometry catheter, it was connected to the manometry system, and the responsiveness of the sensors was then tested by sequentially occluding each of the four catheter openings and observing a sharp upstroke on the pressure tracing. Failure to observe such a response was possibly due to residual air bubbles in the catheter or the manometry system that needed to be evacuated. Subsequently, the system was calibrated at 0 and 50 cm levels relative to the transducer. The catheter was then passed through the snout of the pig guided by direct visualization through the mouth using a laryngoscope. To ensure that the catheter was not coiled in the oropharynx, it was advanced into the esophagus using a Magill forceps until the four sensors were in the stomach. Using a stationary pull-back technique, the catheter was manually pulled out with a speed of 5 mm/min through the lower sphincter of the esophagus (LES), with registration of the pressure within the LES by all four sensors. It was absolutely crucial to establish gastric and esophageal baseline pressures on either side of the LES and to recognize the respiratory inversion point within the LES. The lower border of the LES was determined at the station that demonstrated a consistent rise of pressure above the gastric baseline pressure, whereas the upper border of the LES was determined at the station that showed a drop of pressure to esophageal baseline pressure. Before removing the catheter, the distance between the upper border of the LES and the snout was measured for the subsequent insertion of the pH probe. This measurement was repeated three times and the mean values were recorded to allow for more accurate readings. From the data obtained, which were automatically recorded as pressure curves via a polygraph

on a standard PC using GastroTrac (version 4.3.0.47, Alpine Biomed ApS, Skovlunde, Denmark), we measured the total length, abdominal length, and resting pressure of the LES, as previously described by Zaninotto et al [66]. All three manometric measurements were performed in every experimental animal before and 2 weeks after cardiomyectomy as well as at 6 weeks after injection of DxHA or Saline.

2.5 24-hour ambulatory esophageal pH measurements:

After completing the manometry measurement and under the same general endotracheal anesthesia, a single-sensor, internal reference pH catheter (MUI Scientific, Mississauga, Ontario, Canada) was used to obtain 24-hour pH measurement. Initially, the probe was calibrated using standard pH=1 and pH=7 solutions. Calibration was achieved with a Digitrapper pH 400 (Medtronic, Minneapolis, Minnesota, USA). After successful calibration, the probe was inserted through an adjusted simple intravenous line tube in order to make the probe more rigid to withstand coiling in the nasopharynx. It was very important to ensure that the sensor at the tip of the probe was exposed and not covered with the over-tube to be able to measure pH. Using multiple 2-0 silk sutures, the probe was fixed to the over-tube to prevent the probe from sliding inside the tube especially when the animal was ambulatory. Subsequently, the pH probe was inserted through the pig's snout and guided through the mouth with the aid of a laryngoscope and a Magill forceps, and advanced into the esophagus. A continuous fluoroscopy was used in the first few animals to ensure proper placement of the probe, and that it was not coiling in the chest. However, after the first few insertions, we realized that direct visualization and using the over-tube was sufficient for proper placement. Then, the tip of the probe was placed 2 cm above the manometrically identified upper border of the LES, and marked at

the snout level to allow recognition of changes in its position. The probe was then secured externally with several interrupted 0 silk sutures spaced 5 cm apart, starting from the snout proceeding to the animal's back, leaving enough slack between the sutures to allow the animal freedom of movement without displacing the probe. Finally, a custom-made dorsal harness with a pocket was used to house the Digitrapper. Before awakening the pig, it was of utmost importance to keep the harness tight with several sutures, if needed, simply because the animal would try to remove it once the pig was fully awake. Upon recovery, the animal was allowed free access to water and food in a private cage to avoid disruption of the system by other pigs. After 24 hours, the sutures were cut and probe was removed without the need for any sedation. Data were uploaded to a computer using GastroTrac software (version 4.3.0.47, Alpine Biomed ApS, Skovlunde, Denmark). Acid reflux was defined as a drop in esophageal pH below 5. Data obtained were the number of reflux episodes, number of long refluxes (>5 min), longest reflux episode (min), total time of pH<5 (min), percentage of time pH<5, mean duration of reflux episodes (min). Demeester and Boix-Ochoa scores, modified to pH<5, were calculated.

2.6 Cardiomyectomy:

Following the preparation described and under general anesthesia, the pig was placed in supine position and the abdomen was shaved. Iodine solution was used to disinfect the incision site followed by regular laparotomy draping. Prophylactic antibiotic (cefazolin) was injected intravenously at a dose of 20 mg/kg and intramuscular injection of 0.01 mg/kg Buprenorphine was given for analgesia. A14-french orogastric tube was inserted. The abdomen was accessed through an upper midline laparotomy from the xiphoid to the umbilicus. A self-retaining abdominal retractor was placed to optimize

exposure. The left lobe of liver was gently retracted to the right in order to expose the GEJ. To further improve exposure, the elongated spleen was packed down and to the left. The GEJ was identified with the help of the orogastric tube and the phrenoesophageal membrane was incised. The anterior peritoneal reflection of the oesophagus was incised and the anterior and posterior vagal trunks were identified and preserved. If any of the short gastric vessels were found to be under tension, they were ligated. After full mobilization of the GEJ, a Penrose drain was placed around the lower esophagus to allow manipulation of the GEJ. At the GEJ, an anterior longitudinal myotomy was performed by incising the longitudinal and the circular muscle fibers using a No. 15 blade and a fine Metzenbaum scissors. The myotomy was extended 3 cm caphalad on the esophagus and 3 cm caudad on the stomach (Figure 2). Care was exercised to avoid inadvertent mucosal perforation. Once the submucosal plane was entered, the esophageal muscles were gently peeled away 1 cm on both sides. Both pieces of muscle were then excised creating a myectomy segment that was 6 cm in length (3 cm at the esophageal side and 3 cm at the gastric side) and 2 cm in width centered at the GEJ. An essential part of the myectomy was to ensure dividing the gastric sling and clasp muscle fibers, a step that required careful dissection and carried the highest risk of mucosal perforation. In case of inadvertent perforation, a primary repair with 4-0 absorbable monofilament sutures was done. We found that bleeding from excised muscle edges could be easily controlled with pressure. Once hemostasis was insured, the abdomen was closed in the standard fashion with absorbable monofilament sutures. The skin was closed with subcuticular sutures to avoid the presence of any material externally. OpSite® waterproof spray was used as a dressing. The animal was awakened and extubated. An intramuscular injection of

Buprenorphine 0.01 mg/kg was administered for analgesia. The pigs were allowed free access to food and water, unless mucosal perforation occurred. In that case, water was allowed for 24 hours until the pig was evaluated by the surgeon. Postoperatively, cephalexin (25 mg/kg) was administered orally twice a day for a total of 10 days. In the postoperative period, animals were visited frequently by the surgeon and animal health technician to evaluate their appetite, weight gain, wound, and their fecal and urine output. Two weeks after surgery, the animals underwent manometry and 24-hour pH measurements as described earlier.

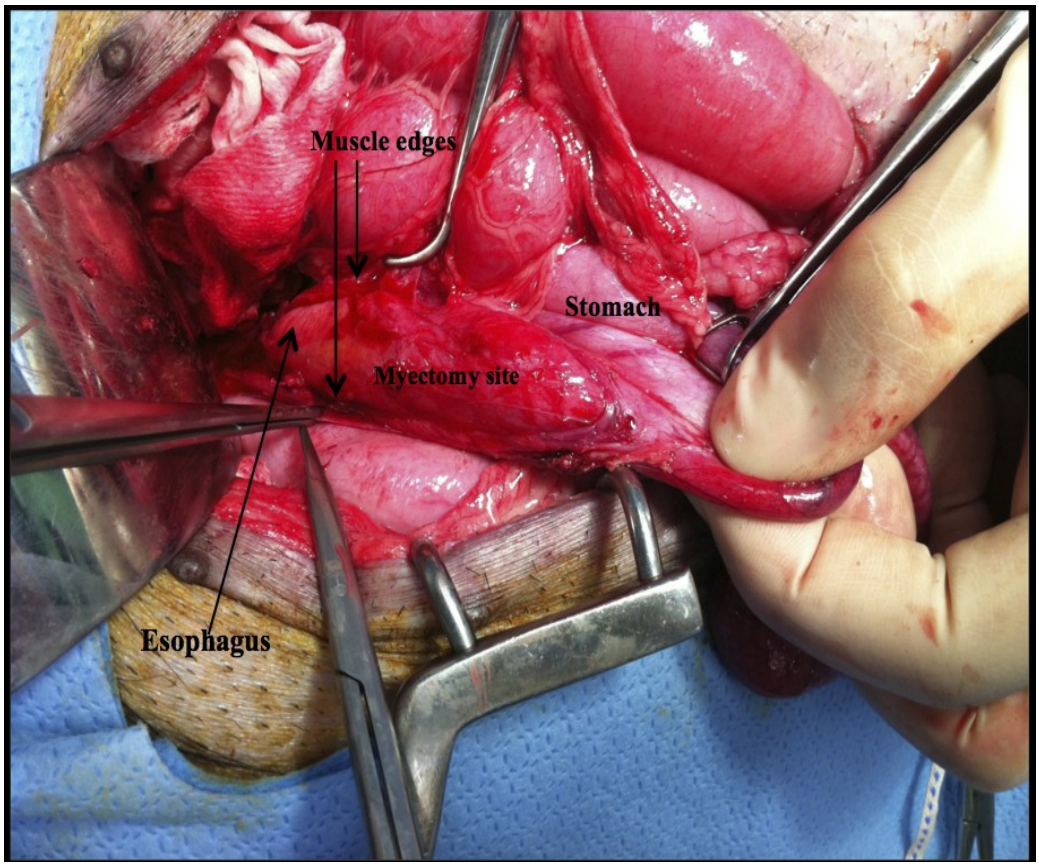

Figure 2: Appearance of the completed myectomy.

2.7 Endoscopic injections:

A 45 cm custom-made rigid endoscope was designed with the help of an engineer (Fiegert-Endotech, Sunrise, Florida, USA). A sheath, made from stainless steel, was attached to the 30 ° scope and worked as a channel for the needle used for the injections. A 2-week recovery period was allowed after the surgery, during which computer-generated randomization to either DxHA or saline was performed. The animal was then placed under general anesthesia and the scope was advanced through the pig's mouth. After the GEJ was identified, a semi-rigid beveled needle was advanced through a working channel in the scope. Submucosal injections of DxHA or saline were performed in three different quadrants away from the muscle-deficient cardiomyectomy site to avoid injecting outside the esophagus. A volume of 1 ml of DxHA or saline was injected in each site. A 30 second waiting period was allowed for the implant to stabilize before withdrawing the needle. Immediate mucosal bulging indicated successful implantation (Figure 3). Six animals had DxHA injections, whereas four animals received saline injections. After completing the injection session, the animal was awakened and extubated. Free access to water and food was allowed immediately after the procedure.

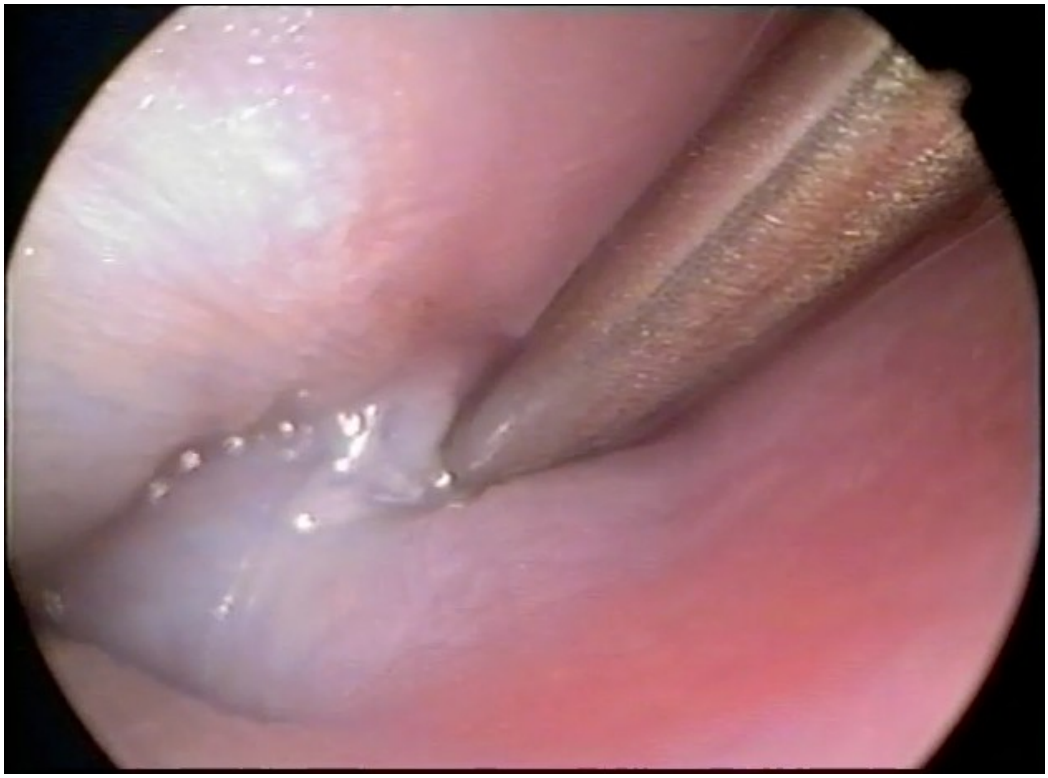

Figure 3: Endoscopic view during injection. Bulging mucosa indicated successful implantation.

2.8 Recovery period:

A 6-week recovery period was allowed after the implantation, during which animals were weighed daily. Their behavior and feeding patterns were recorded. The animal health technicians performed daily assessments, and a veterinarian was consulted upon observing any abnormal signs. At six weeks after injections, the animals underwent a third manometry and 24-hour ambulatory pH measurements.

2.9 Euthanasia and necropsy:

The pigs were sedated via intramuscular injection with a mixture of Butorphanol (0.1 mg/kg), Acepromazine (0.2 mg/kg), and Atropine (0.05 mg/kg). After a minimum of

15 minutes, they were given ketamine (0.14 ml/kg) intramuscularly. Anesthesia was then maintained using 5% isoflurane gas, and subsequently an intravenous injection of sodium pentobarbital at a dosage of 120 mg/kg was given for euthanasia. Upon confirmation of death by animal health technician, necropsy was performed through a midline sternotomy and laparotomy. En bloc resection of esophagus, stomach, liver, and lungs was performed. Specimens were kept in formalin and sent to the pathologists.

2.10 Histological assessment:

Gross and histological examinations were performed by two pathologists. Several sections were made from the GEJ at different levels and stained with Hematoxylin-Phloxine-Saffron stain. Specimens were examined for response to the injection within the GEJ, type of cellular reaction, signs of implant migration, reaction outside the esophagus, stenosis, or perforation. Additionally, liver and lungs were examined for abnormal reaction and evidence of aspiration respectively.

2.11 Statistical analysis:

Weight progression was compared using ANCOVA controlling for the weight at time of injection. Paired continuous data were compared using paired Wilcoxon-signed rank test. Unpaired comparison of continuous data was performed using Mann-Whitney test. P-value of less than 0.05 was considered significant. Analysis was performed using IBM SPSS statistics 20.0 and reviewed by a biostatistician. Pathology results were reported qualitatively.

3.0 Results:

During the study period, two pigs died a few days after performing uneventful cardiomyectomy and prior to randomization. One animal had recurrent vomiting with aspiration pneumonia diagnosed on necropsy. The other pig also died in the postoperative period with signs of acute heart failure of unknown etiology. None of these two animals received endoscopic injection of saline or DxHA, and they were excluded from the analysis. The other 10 pigs survived through the study period without clinical signs of esophageal perforation or obstruction. No allergic reaction was observed due to injections. All pigs continued to gain weight with no significant difference between DxHA or saline groups as determined by ANCOVA (p=0.98) (Figure 4).

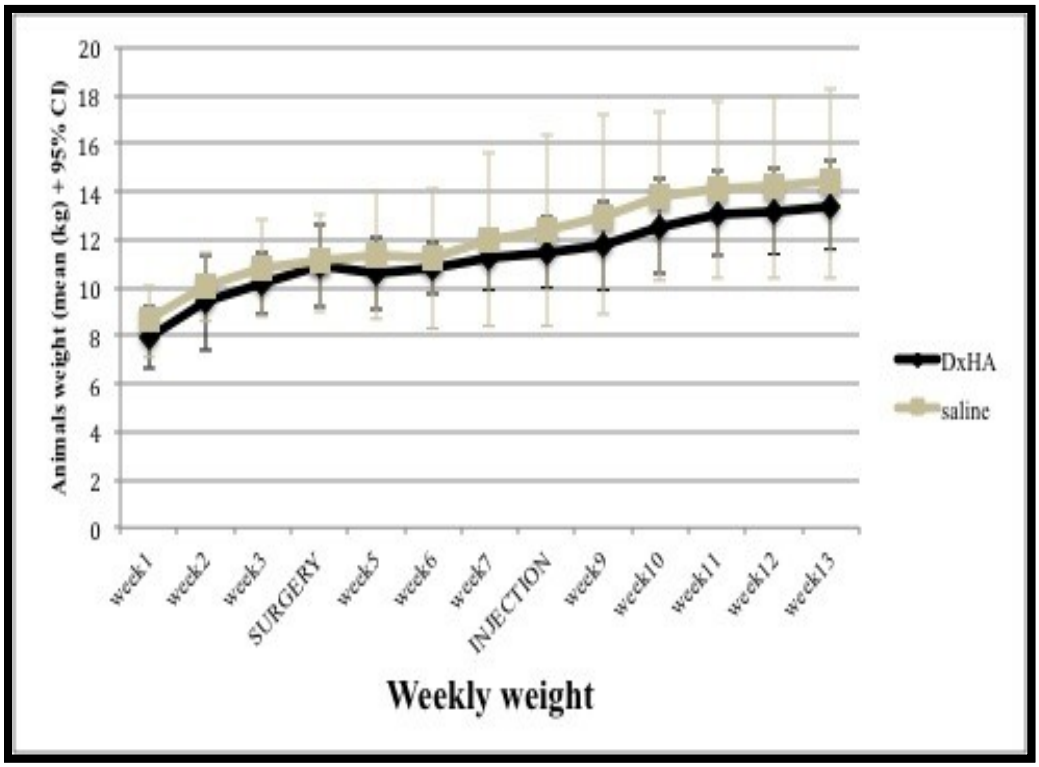

Figure 4: Weekly weight progression of animals in each group.

The effect of cardiomyectomy to induce reflux is shown by comparing manometry and pH measurements pre and post cardiomyectomy using paired Wilcoxon-signed rank test (Table 1). The reductions in LES pressure, total length, and abdominal length were not statistically significant. Surgery resulted in decreasing median LES pressure from 1.35 mmHg (IQR: 1 to 3.03 mmHg) to 1 mmHg (IQR: 0.63 to 1.6 mmHg) (p=0.06). Significantly increased esophageal acid exposure was demonstrated by increase in the percentage of time pH<5 after cardiomyectomy from a median of 0.6% (IQR: 0 to 3%) to 11.6% (IQR: 1.9 to 27.8%) (p=0.02). Furthermore, the total time of pH<5 significantly increased from a median of 9 minutes (IQR: 0 to 39 minutes) to a median of 147 minutes (IQR: 147 to 350 minutes) (p=0.02), and the duration of the longest reflux episode significantly increased from a median of 1 minute (IQR: 0 to 10 minutes) to a median of 28 minutes (IQR: 2 to 64 minutes) (p=0.01). In contrast, changes in the number of refluxes, number of long refluxes (>5 minutes), and mean duration of reflux episodes were not statistically significant. Despite that Demeester score significantly increased after cardiomyectomy from a median of 1.1 (IQR: 0.1 to 7.3) to a median of 11.1 (IQR: 8.3 to 25.8) (p=0.03), Boix-Ochoa score did not significantly change after cardiomyectomy.

	Preoperative data			Postoperative data			P
	Median	25%	75%	Median	25%	75%	
Manometry:							
LES pressure (mmHg)	1.35	1	3.03	1	0.63	1.6	0.06
LES total length (cm)	3.68	2.5	7	2.1	1.75	3.5	0.08
LES abdominal length (cm)	2	1	2.75	1	0.5	1.1	0.07
PH measurement:							
Number of refluxes	10	1	178	218	107	311	0.06
Number of long refluxes	0	0	1	4	0	16	0.05
Time of pH<5 (min)	9	0	39	147	24	350	0.02
Percentage of time pH<5	0.6	0	3	11.6	1.9	27.8	0.02
Mean duration of reflux episodes (min)	0.29	0	0.5	0.8	0.21	1.54	NS
Duration of longest reflux episode (min)	1	0	10	28	2	64	0.01
Demeester score	1.1	0.1	7.3	11.1	8.3	25.8	0.03
Boix-Ochoa score	0.5	0.5	1	1	0.5	20.7	NS

Table 1: Preoperative and postoperative manometry and pH data in 10 pigs, comparison was performed using paired Wilcoxon-signed rank test. (NS: non significant)

When comparing the six pigs in the DxHA group and the four pigs in the saline group, there was no statistically significant difference in either manometry or pH measurements as determined by Mann-Whitney U test (Table 2). In order to control for the difference between baseline values, another comparison was performed between postoperative and postinjection median differences. We noted an increase in LES length in the DxHA compared to saline group (1.8 cm vs. 0.4 cm, p=0.03). This observation was consistent when performing subgroup analysis of DxHA group only (6 pigs) comparing postoperative and postinjection median LES length (1.8 cm vs. 3.3 cm, p=0.06) (Figure 5).

	DxHA			Saline			P
	Median	25%	75%	Median	25%	75%	
Amount of injected agent (ml)	3	2.9	4.5	8	6	12.3	
Manometry:							
LES pressure (mmHg)	0.9	0.8	1.8	0.6	0.5	0.9	NS
LES total length (cm)	3.3	2.7	4.3	3.4	2.9	4.3	NS
LES abdominal length (cm)	1.8	0.9	2.3	1	1	1	NS
PH measurement:							
Number of refluxes	258.5	69	381.5	324	228.5	370	NS
Number of long refluxes	4.5	1.8	13.3	4	0.8	10.3	NS
Time of pH<5 (min)	181.5	95	471.5	158.5	93.3	385	NS
Percentage of time pH<5	12.9	7.1	33.1	11.2	6.8	26.8	NS
Mean duration of reflux episodes (min)	0.9	0.7	1.7	0.7	0.3	1.2	NS
Duration of longest reflux episode (min)	109.5	26	166.3	63	15.8	87	NS
Demeester score	14.2	8.4	30.1	14.6	13.5	23	NS
Boix-Ochoa score	4.1	0.5	35.8	7.3	0.5	56.8	NS

Table 2: Manometry and pH data in DxHA group (6 pigs) and saline group (4 pigs). Comparisons were performed using Mann-Whitney test. (NS: non significant)

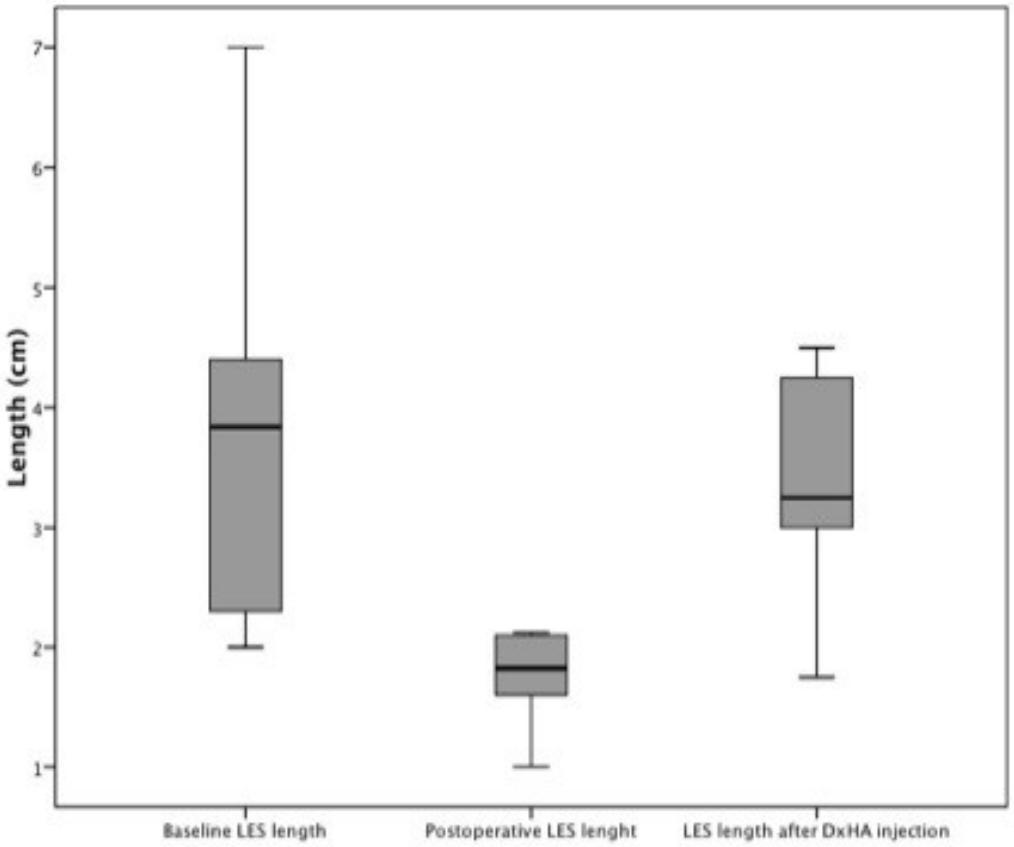

Figure 5: Box plot of the LES total length in the DxHA group at baseline, post cardiomyectomy, and 6 weeks post injection.

We also calculated the percentage of the change in each median value between postoperative and postinjection data sets. In comparison to saline, DxHA resulted in 120% increase in the median LES pressure difference (median change of pressure -0.5 mmHg in saline group, 0.1 mmHg in DxHA group**,** p=0.8), and 124% decrease in the median difference in the number of reflux episodes between both groups (median change in the number of reflux episodes -22 in the DxHA group, +91.5 in the saline group, p=0.6).

The histologic results are shown in Table 3. Gross examination of the GEJ by two pathologists revealed an area of increase thickness with no luminal narrowing in DxHA injected animals. Microscopically, DxHA implants were identified tracking from the submucosa to the muscularis propria of all 6 specimens accompanied by foreign body fibrous tissue reaction with giant cells and collagen deposition (Figure 6). No fibrous tissue reaction was seen in the saline group. In 4 out of 6 DxHA specimens, acute inflammatory cells (micro-abscesses) were identified close to the injection site. No evidence of perforation or peri-esophageal inflammation was noted. DxHA did not migrate above or below the site of injection. There was no histologic evidence of GERD in any of the specimens.

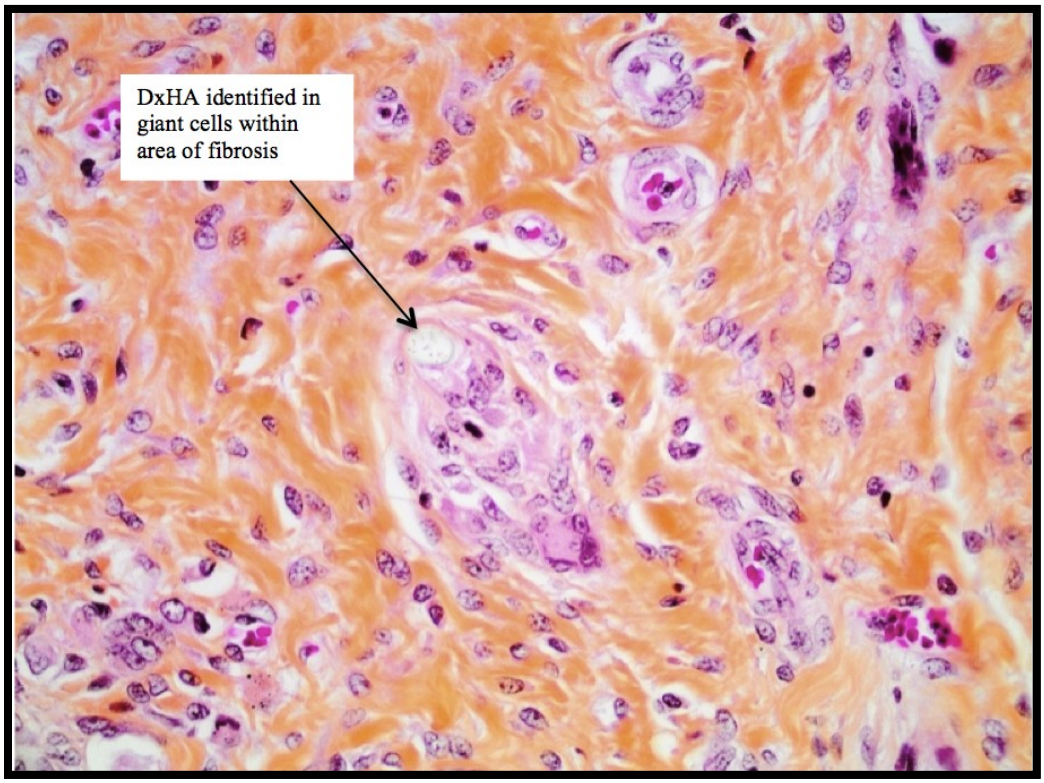

Figure 6: Microscopic examination of the GEJ 6 weeks after DxHA implantation stained with Hematoxylin-Pholxine-Saffron stain.

Pigs	Injection type	DxHA implant presence	Foreign body reaction presence	Giant cells presence	Fibroblasts presence	Micor-abscess	Comment
1	Saline	No	No	No	No	No	No inflammation noted
2	Saline	No	No	No	No	No	No inflammation noted
3	Saline	No	No	No	No	No	No inflammation noted
4	Saline	No	No	No	No	No	No inflammation noted
5	DxHA	Yes	Yes	Yes	Yes	No	DxHA identified outside the muscularis propria
6	DxHA	Yes	Yes	Yes	Yes	Yes	DxHA identified in the muscularis propria. Presence of abscess with a small amount of DxHA outside the muscularis propria
7	DxHA	Yes	Yes	Yes	Yes	No	DxHA tracks outside the muscularis propria
8	DxHA	Yes	Yes	Yes	Yes	Yes	DxHA identified outside the muscularis propria. Abscess identified in the submucosa
9	DxHA	Yes	Yes	Yes	Yes	Yes	DxHA identified in the muscularis propria
10	DxHA	Yes	Yes	Yes	Yes	Yes	DxHA present within a 4 cm abscess located outside the muscularis propria and near the myomectomy site

Table 3: Histology results

Discussion:

GERD is a very common condition affecting both children and adults with an emerging evidence that it's prevalence is rising. In Western populations, the prevalence of GERD is estimated to be 10-20% in children and adults [1,67-71]. GERD is an established risk factor for Barrett's esophagus and esophageal adenocarcinoma [72,73]. Recent reports have shown rising trends in the incidence rates of esophageal adenocarcinoma in the United States that coincide with increased prevalence of GERD [74]. It has been established that symptoms of GERD adversely affect health-related quality of life (HR-QOL), and result in significant cost to the health care system [8,75,76]. In 2009, GERD was the most frequent outpatient diagnosis with almost 9 million visits in the United States, with an estimated cost of US $390 million per year [77]. Medical treatment in the form of acid suppressing medications, primarily PPIs, are typically offered to the patients with variable success rates particularly those with severe GERD or underlying neuromuscular disorders [14,19,23,25,78,79]. Despite the efficacy of PPIs in controlling GERD-related symptoms, high rates of recurrence are observed once treatment is discontinued [15,18,19,23,80]. Recently, the safety of these agents, particularly PPIs, has been questioned as increased risks of community acquired pneumonia, gastroenteritis, *Clostridium difficile* colitis, and hypomagnesemia have been observed [19,26,27,81-84]. ARS, namely fundoplication, is commonly offered to patients with refractory or complicated GERD. Outcomes of ARS have been recently challenged since a large proportion of patients remains on PPIs after the surgery [21,29,30,32,33,35,36,39,40,85-87]. As a result, extensive research for an effective alternative treatment has been conducted over the past few decades with mixed results

[47-52,54]. Augmenting the LES with polymer injections has been investigated as a potential anti-GERD strategy [47,49-53,55]. Some of these polymers were synthetic, while others were biocompatible. Enteryx® is a liquid agent, composed of ethylene vinyl alcohol and dimethyl sulfoxide, that consolidates after being endoscopically injected at the GEJ. Initial results in animal studies and adult patients were promising [51,53]. However, serious side effects resulted from improper placement of this bulking agent. Intra-aortic injection of Enteryx® caused aortoenteric fistula and renal failure from arterial occlusion leading to its voluntary recall in 2005 [88-90]. Another injectable agent, Plexiglas®, consists of a suspension of polymethylmethacylate microspheres in gelatin solution. Once injected, it is phagocytized by macrophages and subsequently replaced by fibroblasts and collagen. A significant decrease in both GERD symptoms and esophageal acid exposure were achieved. However, this agent was also withdrawn [52,91]. To our knowledge, there is currently no injectable agent approved for the treatment of GERD.

Nevertheless, there is still strong evidence that augmentation of the LES pressure alone may be an effective anti-reflux measure. Ganz et al. recently reported three-year outcomes of a flexible magnetic ring used to augment the LES pressure, showing an effective treatment of GERD in most patients [92]. This device exerts its effect exclusively by augmenting LES pressure. DxHA represents a potentially effective injectable agent because of its appealing properties. Its main component, hyaluronic acid, is a naturally present compound that is non-immunogenic. Studies have shown that DxHA does not migrate from the original site up to 3 years after injection [93]. A granulomatous inflammatory reaction and fibrotic encapsulation likely contribute to the

durability of the implant long after DxHA is hydrolyzed by the body [57]. DxHA has been effectively used for more than two decades in the treatment of VUR. More recently, DxHA was introduced in a variety of other pediatric applications including, closure of recurrent tracheoesophageal fistula [63], closure of umbilical hernia [64], and neuropathic urinary incontinence [94]. In adults, transanal submucosal injection of DxHA has been shown to be effective for the management of fecal incontinence [95]. This application is pertinent to our current work. Whereas DxHA injection in the bladder is thought to work by changing the vesicoureteral angle, injection in the anal sphincter is thought to be effective due to higher sphincter pressure.

In previous work, we have found that injection of DxHA in the rabbit GEJ was well tolerated and did not result in esophageal obstruction or perforation. Histologically, DxHA was able to induce fibrous tissue reaction at GEJ similar to reaction observed in ureterovesical junction [65]. The rabbit foregut anatomy is remarkably similar to humans. However, despite several attempts, we were not able to create a reliable GERD model in the rabbit. The muscular layer of the GEJ in the rabbit is quite thin, rendering myectomy without perforation very difficult. The rabbit esophagus is narrow, and endoscopic injection was not possible. In addition, the rabbit's stomach never completely empties and always contains large bezoars from ingested hair. Ambulatory pH measurements in rabbits were not possible.

Several GERD large animal models have been investigated with the aim to further understand diseases of the GEJ such as GERD, Barrett's oesophagus, non-acid reflux, or achalasia. However, there is a lack of a standardized and reproducible GERD model. Furthermore, no consensus exists on the ideal operative technique to induce GERD. Elton

et al showed that cardiomyectomy is superior to cardiomyotomy performed on rabbits with respect to the degree of acid reflux [96]. When compared to partial cardiomyectomy in rats, total cardiomyectomy resulted in a higher animal loss rate with similar degree of reflux [97]. Dogs have been used with good results. However, their GEJ should be approached through the chest because of their short abdominal esophagus [98-102]. We developed our animal model based on pilot work by Schopf et al [102]. We learned important lessons during development of our model. The porcine GEJ is quite complex because of well-developed clasp and sling oblique gastric fibers that act as a natural partial fundoplication, a phenomenon that was previously observed by Vicente et al [103]. These fibers have to be divided to induce GERD. We found the dissection of the GEJ by laparoscopy to be quite difficult due to the ease of entering the pleura and causing pneumothorax. In addition, an adequate myectomy required significant mobilization of the GEJ and deliberate division of each muscular layer, which we found significantly more involved than in human operations. Nevertheless, others have reported achieving the same goal in pigs by laparoscopy [99]. Interestingly, despite a generous myectomy, we found identification of the myectomy site quite difficult on subsequent endoscopy. This may have prevented us from injecting exactly at the myectomy site, and may have influenced our results. Although quite involved, our procedure of 24-hour ambulatory pH monitoring in pigs was possible and reproducible

This study demonstrates the feasibility and safety of DxHA implantation into the GEJ in a porcine GERD model. Implantation of DxHA at the GEJ is technically feasible using a rigid scope with a beveled needle. However, the tip of the needle must not traverse the esophageal wall. This may have occurred in some of our injections, and may

explain the micro-abscesses seen in some animals. More accurate injection may be facilitated by the use of a needle with limited extension, such as a sclerotherapy needle, or by employing endoscopic ultrasound. All injected animals survived and thrived without any evidence of esophageal obstruction or perforation. Functional studies (manometry and pH measurements) performed 6 weeks after implantation of DxHA suggested augmentation of the GEJ and decrease in the degree of reflux. In particular, the length of the high-pressure zone, namely the LES, significantly increased in the DxHA group. Grossly, the implants were not identified. Nevertheless, there was obvious thickening at the GEJ probably due to the reaction induced by DxHA. Microscopic examination of the GEJ revealed that DxHA implants were durable and able to incite a predictable granulomatous fibrous tissue reaction as early as 6 weeks after implantation.

It should be emphasized that this is a small pilot study that does not conclusively offer data supporting a therapeutic role for DxHA in the treatment of GERD. The small sample size in each arm and the short survival period limit the power of the study. In addition, no animal model can duplicate the pathophysiology of human GERD. However, the data presented here continues to raise the possibility of a therapeutic role for this agent in GERD in both children and adults. In future work, appropriate sample size should be calculated. Also, multiple arms might be necessary to examine the dose-response relationship. Longer survival period may be needed to investigate the persistence of the effect of DxHA implantation. In addition, further refinements in the experimental techniques are necessary in order to produce stronger evidence and potentially allow for a translational research in patients through a limited trial.

References

1. Nelson SP, Chen EH, Syniar GM, et al: Prevalence of symptoms of gastroesophageal reflux during childhood: a pediatric practice-based survey. Arch Pediatr Adolesc Med 2000;154:150-154.

2. Fonkalsrud EW, Ament ME: Gastroesophageal reflux in childhood. Curr Probl Surg 1996;33:1-70.

3. Tougas G, Chen Y, Pieway HPD, et al: Prevalence and impact of upper gastrointestinal symptoms in the Canadian population: findings from the DIGEST study. Am J Gastroenterol 1999;94:2845-2854.

4. Camilleri M, Dubois D, Coulie B, et al: Prevalence and socioeconomic impact of upper gastrointestinal disorders in the United States: results of the US Upper Gastrointestinal Study. Clin Gastroenterol Hepatol 2005;3:543-552.

5. Farup C, Kleinman L, Sloan S, et al: The impact of nocturnal symptoms associated with gastroesophageal reflux disease on health-related quality of life. Arch Intern Med 2001;161:45-52.

6. Eloubeidi MA, Provenzale D: Health-related quality of life and severity of symptoms in patients with Barrett's esophagus and gastroesophageal reflux disease patients without Barrett's esophagus. Am J Gastroenterol 2000;95:1881-1887.

7. Kappelman MD, Dorn SD, Peterson E, et al: Quality of care for gastrointestinal conditions: a primer for gastroenterologists. Am J Gastroenterol 2011;106:1182-1187.

8. Shin WG, Kim HU, Kim SG, et al: Work productivity and activity impairment in gastroesophageal reflux disease in Korean full-time employees: A multicentre study. Dig Liver Dis 2012;44:286-291.

9. Fedorak RN, Veldhuyzen van Zanten S, Bridges R: Canadian Digestive Health Foundation Public Impact Series: gastroesophageal reflux disease in Canada: incidence, prevalence, and direct and indirect economic impact. Can J Gastroenterol 2010;24:431-434.

10. Wiklund I: Review of the quality of life and burden of illness in gastroesophageal reflux disease. Dig Dis 2004;22:108-114.

11. Wahlqvist P, Reilly M, Barkun A: Systematic review: the impact of gastro-oesophageal reflux disease on work productivity. Aliment Pharmacol Ther 2006;24:259-272.

12. Marlais M, Fishman JR, Koglmeier J, et al: Reduced quality of life in children with gastro-oesophageal reflux disease. Acta Paediatr 2010;99:418-421.

13. Armstrong D, Marshall JK, Chiba N, et al: Canadian Consensus Conference on the management of gastroesophageal reflux disease in adults - update 2004. Can J Gastroenterol 2005;19:15-35.

14. Belousov Iu V: Gastroesophageal reflux disease in childhood. Eksp Klin Gastroenterol 2011;64-71.

15. Hershcovici T, Fass R: Pharmacological management of GERD: where does it stand now? Trends Pharmacol Sci 2011;32:258-264.

16. Van Pinxteren B, Numans M, Bonis P, et al: Short-term treatment with proton pump inhibitors, H2-receptor antagonists and prokinetics for gastro-oesophageal reflux disease-like symptoms and endoscopy negative reflux disease. Cochrane Database Syst Rev. 2006;3:CD002095.

17. Pohle T, Domschke W: Results of short-and long-term medical treatment of gastroesophageal reflux disease (GERD). Langenbecks Arch Surg 2000;385:317-323.

18. van der Pol RJ, Smits MJ, van Wijk MP, et al: Efficacy of proton-pump inhibitors in children with gastroesophageal reflux disease: a systematic review. Pediatrics 2011;127:925-935.

19. Tjon JA, Pe M, Soscia J, et al: Efficacy and Safety of Proton Pump Inhibitors in the Management of Pediatric Gastroesophageal Reflux Disease. Pharmacotherapy 2013 May 26. doi: 10.1002/phar.1229. [Epub ahead of print]

20. Romano C, Chiaro A, Comito D, et al: Proton pump inhibitors in pediatrics: evaluation of efficacy in GERD therapy. Curr Clin Pharmacol 2011;6:41-47.

21. Lee SL, Sydorak RM, Chiu VY, et al: Long-term antireflux medication use following pediatric Nissen fundoplication. Arch Surg 2008;143:873.

22. Lightdale JR, Gremse DA, Heitlinger LA, et al: Gastroesophageal Reflux: Management Guidance for the Pediatrician. Pediatrics 2013;131:e1684-e1695.

23. Winter H, Gunasekaran T, Tolia V, et al: Esomeprazole for the Treatment of GERD in Infants Ages 1–11 Months. J Pediatr Gastroenterol Nutr 2012;55:14-20.

24. Winter H, Kum-Nji P, Mahomedy SH, et al: Efficacy and safety of pantoprazole delayed-release granules for oral suspension in a placebo-controlled treatment-withdrawal study in infants 1-11 months old with symptomatic GERD. J Pediatr Gastroenterol Nutr 2010;50:609-618.

25. Gilger M, Tolia V, Vandenplas Y, et al: Safety and tolerability of esomeprazole in children with gastroesophageal reflux disease. J Pediatr Gastroenterol Nutr 2006;43:E20-E21.

26. Laheij RJ, Sturkenboom MC, Hassing R-J, et al: Risk of community-acquired pneumonia and use of gastric acid–suppressive drugs. JAMA 2004;292:1955-1960.

27. Gold BD, Gunasekaran T, Tolia V, et al: Safety and symptom improvement with esomeprazole in adolescents with gastroesophageal reflux disease. J Pediatr Gastroenterol Nutr 2007;45:520-529.

28. Mauritz FA, van Herwaarden-Lindeboom MY, Stomp W, et al: The effects and efficacy of antireflux surgery in children with gastroesophageal reflux disease: a systematic review. J Gastrointest Surg 2011;15:1872-1878.

29. Orenstein SR, Carlo Di Lorenzo M: Postfundoplication complications in children. Curr Treat Options Gastroenterol 2001;4:441-449.

30. Lee SL, Shabatian H, Hsu J-W, et al: Hospital admissions for respiratory symptoms and failure to thrive before and after Nissen fundoplication. J Pediatr Surg 2008;43:59-65.

31. Kawahara H, Okuyama H, Kubota A, et al: Can laparoscopic antireflux surgery improve the quality of life in children with neurologic and neuromuscular handicaps? J Pediatr Surg 2004;39:1761-1764.

32. Wong KK, Liu XL: Perioperative and late outcomes of laparoscopic fundoplication for neurologically impaired children with gastro-esophageal. Chin Med J (Engl) 2012;125:3905-3908.

33. Esposito C, Montupet P, van Der Zee D, et al: Long-term outcome of laparoscopic Nissen, Toupet, and Thal antireflux procedures for neurologically normal children with gastroesophageal reflux disease. Surg Endosc 2006;20:855-858.

34. Loots C, van Herwaarden MY, Benninga MA, et al: Gastroesophageal Reflux, Esophageal Function, Gastric Emptying, and the Relationship to Dysphagia before and after Antireflux Surgery in Children. J Pediatr 2013;162:566-573.

35. O'Loughlin EV, Somerville H, Shun A, et al: Antireflux Surgery in Children With Neurological Impairment: Caregiver Perceptions and Complications. J Pediatr Gastroenterol Nutr 2013;56:46-50.

36. Shariff F, Kiely E, Curry J, et al: Outcome after laparoscopic fundoplication in children under 1 year. J Laparoendosc Adv Surg Tech A 2010;20:661-664.

37. McHoney M, Wade AM, Eaton S, et al: Clinical outcome of a randomized controlled blinded trial of open versus laparoscopic Nissen fundoplication in infants and children. Ann Surg 2011;254:209-216.

38. Knatten CK, Fyhn TJ, Edwin B, et al: Thirty-day outcome in children randomized to open and laparoscopic Nissen fundoplication. J Pediatr Surg 2012;47:1990-1996.

39. Kane TD, Brown MF, Chen MK: Position paper on laparoscopic antireflux operations in infants and children for gastroesophageal reflux disease. J Pediatr Surg 2009;44:1034-1040.

40. Galmiche J-P, Hatlebakk J, Attwood S, et al: Laparoscopic antireflux surgery vs esomeprazole treatment for chronic GERD. JAMA 2011;305:1969.

41. Mahon D, Rhodes M, Decadt B, et al: Randomized clinical trial of laparoscopic Nissen fundoplication compared with proton- pump inhibitors for treatment of chronic gastro- oesophageal reflux. Br J Surg 2005;92:695-699.

42. Spechler SJ, Lee E, Ahnen D, et al: Long-term outcome of medical and surgical therapies for gastroesophageal reflux disease. JAMA 2001;285:2331-2338.

43. Ciovica R, Michael Gadenstätter M, Klingler A, et al: Quality of life in GERD patients: medical treatment versus antireflux surgery. J Gastrointest Surg 2006;10:934-939.

44. Lundell L, Miettinen P, Myrvold H, et al: Continued (5-year) followup of a randomized clinical study comparing antireflux surgery and omeprazole in gastroesophageal reflux disease. J Am Coll Surg 2001;192:172-179.

45. Lundell L, Miettinen P, Myrvold H, et al: Seven- year follow- up of a randomized clinical trial comparing proton- pump inhibition with surgical therapy for reflux oesophagitis. Br J Surg 2007;94:198-203.

46. Bammer T, Klaus A, Klingler PJ: Five-to eight-year outcome of the first laparoscopic Nissen fundoplications. J Gastrointest Surg 2001;5:42-48.

47. Arts J, Tack J, Galmiche J: Endoscopic antireflux procedures. Gut 2004;53:1207.

48. Aziz AM, El-Khayat HR, Sadek A, et al: A prospective randomized trial of sham, single-dose Stretta, and double-dose Stretta for the treatment of gastroesophageal reflux disease. Surg Endosc 2010;24:818-825.

49. Chen D, Barber C, McLoughlin P, et al: Systematic review of endoscopic treatments for gastro-oesophageal reflux disease. Br J Surg 2009;96:128-136.

50. Devière J, Costamagna G, Neuhaus H, et al: Nonresorbable copolymer implantation for gastroesophageal reflux disease: a randomized sham-controlled multicenter trial. Gastroenterology 2005;128:532-540.

51. Johnson DA, Ganz R, Aisenberg J, et al: Endoscopic implantation of enteryx for treatment of GERD: 12-month results of a prospective, multicenter trial. Am J Gastroenterol 2003;98:1921-1930.

52. Kamler JP, Lemperle G, Lemperle S, et al: Endoscopic lower esophageal sphincter bulking for the treatment of GERD: safety evaluation of injectable polymethylmethacrylate microspheres in miniature swine. Gastrointest Endosc 2010;72:337-342.

53. Mason RJ, Hughes M, Lehman GA, et al: Endoscopic augmentation of the cardia with a biocompatible injectable polymer (Enteryx) in a porcine model. Surg Endosc 2002;16:386-391.

54. Pleskow D, Rothstein R, Lo S, et al: Endoscopic full-thickness plication for the treatment of GERD: a multicenter trial. Gastrointest Endosc 2004;59:163-171.

55. Spicak J: Treatment of gastroesophageal reflux disease: endoscopic aspects. Dig Dis 2007;25:183-187.

56. Utley DS, Kim M, Vierra MA, et al: Augmentation of lower esophageal sphincter pressure and gastric yield pressure after radiofrequency energy delivery to the gastroesophageal junction: a porcine model. Gastrointest Endosc 2000;52:81-86.

57. Stenberg A, Larsson E, Läckgren G: Endoscopic treatment with dextranomer-hyaluronic acid for vesicoureteral reflux: histological findings. J Urol 2003;169:1109-1113.

58. Heel R, Morton P, Brogden R, et al: Dextranomer: a review of its general properties and therapeutic efficacy. Drugs 1979;18:89-102.

59. Scarpa AA, Rossi C, Casolari E, et al: Endoscopic treatment of vesicoureteric reflux in paediatric patients: results of a long-term study. Acta Bio Medica Atenei Parmensis 2011;82:137-145.

60. Inglefield C: Early clinical experience of hyaluronic acid gel for breast enhancement. J Plast Reconstr Aesthet Surg 2011;64:722-729.

61. Schwandner O, Brunner M, Dietl O: Quality of Life and Functional Results of Submucosal Injection Therapy Using Dextranomer Hyaluronic Acid for Fecal Incontinence. Surg Innov 2011;18:130.

62. Sood R, Kathuria S: POS-01.126: Initial experience of transurethral bulking agent (deflux injection) in patients presenting with incontinence after radical prostatectomy or in advanced ca prostate involving posterior urethra/sphincter and unresponsive to conservative measures. Urology 2007;70:229.

63. Briganti V, Coletta R, Giannino G, et al: Usefulness of dextranomer/hyaluronic acid copolymer in bronchoscopic treatment of recurrent tracheoesophageal fistula in children. Int J Pediatr Otorhinolaryngol 2011;75:1191-1194.

64. Feins NR, Dzakovic A, Papadakis K: Minimally invasive closure of pediatric umbilical hernias. J Pediatr Surg 2008;43:127-130.

65. Martin K, Emil S, Bernard C, et al: Dextranomer hyaluronic acid copolymer effects on the gastroesophageal junction: A pilot study in rabbits. Presented at the joint meeting of the British Association of Paediatric Surgeons and the European Paediatric Surgical Association, Rome, Italy, June 14-16, 2012.

66. Zaninotto G, DeMeester TR, Schwizer W, et al: The lower esophageal sphincter in health and disease. Am J Surg 1988;155:104-111.

67. Vakil N: Disease definition, clinical manifestations, epidemiology and natural history of GERD. Best Pract Res Clin Gastroenterol 2010;24:759-764.

68. Nelson S, Kothari S, Wu E, et al: Pediatric gastroesophageal reflux disease and acid-related conditions: trends in incidence of diagnosis and acid suppression therapy. J Med Econ 2009;12:348-355.

69. Nelson SP, Chen EH, Syniar GM, et al: Prevalence of symptoms of gastroesophageal reflux during infancy: a pediatric practice-based survey. Arch Pediatr Adolesc Med 1997;151:569-572.

70. Ness-Jensen E, Lindam A, Lagergren J, et al: Changes in prevalence, incidence and spontaneous loss of gastro-oesophageal reflux symptoms: a prospective population-based cohort study, the HUNT study. Gut 2012;61:1390-1397.

71. Nilsson M, Johnsen R, Ye W, et al: Prevalence of gastro-oesophageal reflux symptoms and the influence of age and sex. Scand J Gastroenterol 2004;39:1040-1045.

72. Lagergren J, Bergström R, Lindgren A, et al: Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. N Engl J Med 1999;340:825-831.

73. Kim R, Weissfeld JL, Reynolds JC, et al: Etiology of Barrett's metaplasia and esophageal adenocarcinoma. Cancer Epidemiol Biomarkers Prev 1997;6:369-377.

74. Simard EP, Ward EM, Siegel R, et al: Cancers with increasing incidence trends in the United States: 1999 through 2008. CA Cancer J Clin 2012;62:118-128.

75. Darba J, Kaskens L, Plans P, et al: Epidemiology and societal costs of gastroesophageal reflux disease and Barrett's syndrome in Germany, Italy and Spain. Expert Rev Pharmacoecon Outcomes Res 2011;11:225-232.

76. Raman A, Sternbach J, Babajide A, et al: When does testing for GERD become cost effective in an integrated health network? Surg Endosc 2010;24:1245-1249.

77. Peery AF, Dellon ES, Lund J, et al: Burden of gastrointestinal disease in the United States: 2012 update. Gastroenterology 2012;143:1179-1187.

78. Acierno SP, Chilcote HC, Edwards TC, et al: Development of a quality of life instrument for pediatric gastroesophageal reflux disease: qualitative interviews. J Pediatr Gastroenterol Nutr 2010;50:486-492.

79. Vandenplas Y, Rudolph CD, Di Lorenzo C, et al: Pediatric gastroesophageal reflux clinical practice guidelines: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN). J Pediatr Gastroenterol Nutr 2009;49:498-547.

80. Karoui S, Haddad W, Chebbi F, et al: Long-term functional outcome of laparoscopic anti reflux surgery. Tunis Med 2011;89:693-698.

81. Eurich DT, Sadowski CA, Simpson SH, et al: Recurrent community-acquired pneumonia in patients starting acid-suppressing drugs. Am J Med 2010;123:47-53.

82. Pettit M: Treatment of gastroesophageal reflux disease. Pharm World Sci 2005;27:432-435.

83. Herzig SJ, Howell MD, Ngo LH, et al: Acid-suppressive medication use and the risk for hospital-acquired pneumonia. JAMA 2009;301:2120-2128.

84. Kwok CS, Arthur AK, Anibueze CI, et al: Risk of clostridium difficile infection with acid suppressing drugs and antibiotics: meta-analysis. Am J Gastroenterol 2012;107:1011-1019.

85. Ciovica R, Gadenstatter M, Klingler A, et al: Quality of life in GERD patients: medical treatment versus antireflux surgery. J Gastrointest Surg 2006;10:934-939.

86. Lasser MS, Liao JG, Burd RS: National trends in the use of antireflux procedures for children. Pediatrics 2006;118:1828-1835.

87. Di Lorenzo C, Orenstein S: Fundoplication: friend or foe? J Pediatr Gastroenterol Nutr 2002;34:117-124.

88. Melvin ES, Eakin JL: Endoscopic Considerations in Gastroesophageal Reflux Disease, in Marks JM, Dunkin BJ: Principles of Flexible Endoscopy for Surgeons. New York, Springer, 2013, pp 157-166

89. Noh KW, Loeb DS, Stockland A, et al: Pneumomediastinum following Enteryx™ injection for the treatment of gastroesophageal reflux disease. Am J Gastroenterol 2005;100:723-726.

90. Tintillier M, Chaput A, Kirch L, et al: Esophageal abscess complicating endoscopic treatment of refractory gastroesophageal reflux disease by Enteryx injection: a first case report. Am J Gastroenterol 2004;99:1856-1858.

91. Feretis C, Benakis P, Dimopoulos C, et al: Endoscopic implantation of Plexiglas (PMMA) microspheres for the treatment of GERD. Gastrointest Endosc 2001;53:423-426.

92. Ganz RA, Peters JH, Horgan S, et al: Esophageal sphincter device for gastroesophageal reflux disease. N Engl J Med 2013;368:719-727.

93. McMann LP, Scherz HC, Kirsch AJ: Long-term preservation of dextranomer/hyaluronic acid copolymer implants after endoscopic treatment of vesicoureteral reflux in children: a sonographic volumetric analysis. J Urol 2007;177:316-320.

94. Misseri R, Casale AJ, Cain MP, et al: Alternative uses of dextranomer/hyaluronic acid copolymer: the efficacy of bladder neck injection for urinary incontinence. J Urol 2005;174:1691-1694.

95. Graf W, Mellgren A, Matzel KE, et al: Efficacy of dextranomer in stabilised hyaluronic acid for treatment of faecal incontinence: a randomised, sham-controlled trial. Lancet 2011;377:997-1003.

96. Elton C, Uppal R, Moore D, et al: Partial cardiomyectomy--an animal model of gastro-oesophageal reflux. Int J Surg Investig 1999;1:81-85.

97. Gaia Filho EV, Goldenberg A, Costa HO: Experimental model of gastroesophageal reflux in rats. Acta Cir Bras 2005;20:437-444.

98. Gawad KA, Wachowiak R, Rempf C, et al: Ambulatory long-term pH monitoring in pigs. Surg Endosc 2003;17:1556-1560.

99. Huttl TP, Huttl TK, Lang RA, et al: Laparoscopic partial myectomy: an experimental reflux model. Surg Endosc 2006;20:665-672.

100. McMahon RL, Ali A, Chekan EG, et al: A canine model of gastroesophageal reflux disease (GERD). Surg Endosc 2002;16:67-74.

101. Nino G, Savchenko O, Vastola P, et al: Lipid laden macrophage indices and reflux finding score in canine gastroesophageal reflux model. Pediatr Pulmonol 2007;42:1181-1186.

102. Schopf BW, Blair G, Dong S, et al: A Porcine Model of Gastroesophageal Reflux. J Invest Surg 1997;10:105-114.

103. Vicente Y, da Rocha C, Yu J, et al: Architecture and function of the gastroesophageal barrier in the piglet. Dig Dis Sci 2001;46:1899-1908.