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Endoscopic Muscle Biopsy (EMB) of the Duodenum and Rectum: A Pilot Survival Study in a Porcine Model to Detect Myenteric Neurons

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Abstract

Background—Small bowel and colorectal muscle biopsies require a surgical approach.

Advancing our understanding of the pathophysiology of motility disorders, such as functional bowel disorders, intestinal pseudo-obstruction and slow transit constipation, is hindered by our inability to non-invasively obtain muscularis propria (MP) for evaluation of multiple cell types, including myenteric neurons.

Aims—The aims of this study were to determine: 1) technical feasibility, reproducibility and safety of performing duodenal endoscopic muscle biopsy (dEMB) and rectal endoscopic muscle biopsy (rEMB) using a clip-assist technique; and 2) presence of myenteric neurons in tissue samples.

Methods—Five 40-kg pigs were studied. Each animal underwent a dEMB and rEMB procedure. dEMB was performed using a single resection clip-assist technique. An over-the-scope clip was advanced to the duodenum. Tissue was suctioned into the cap and the clip deployed. The pseudopolyp of duodenal wall created was then resected using snare electrocautery. rEMB was performed using a double resection clip-assist technique. Endoscopic mucosal resection (EMR) was initially performed to uncover the underlying MP using a band ligation technique. An over-the-scope clip was then advanced to the exposed MP. The MP was retracted and suctioned into the cap, and the clip deployed. The pseudopolyp of MP was resected using snare electrocautery. An antibody to Protein Gene Product (PGP) 9.5 was used to determine the presence of myenteric neurons in the samples. Animals were survived for 2 weeks, at which time an upper endoscopy and necropsy were performed.

Results—dEMB and rEMB were successfully performed in all animals with no procedural adverse events using this ‘no hole’ (close then cut) approach. Mean procedure times for dEMB and rEMB were 23.7 ± 2.5 minutes and 13.25 ± 2.8 minutes, respectively. Mean length of resected full thickness duodenal wall was 13.25 ± 4.3 mm and rectal MP was 12.5 ± 1.7 mm. Hematoxylin and Eosin (H&E) stain and antibody to PGP 9.5 confirmed the presence of MP with inner circular,

outer longitudinal and intermuscular layers, including myenteric neurons, in all samples. Clinical course was uneventful in all animals. Repeat upper endoscopy at 2 weeks showed well-healed dEMB sites. Necropsy in all animals showed no perforation, fluid collection or abscess at the dEMB and rEMB sites.

Conclusions—Based on this preclinical study, dEMB and rEMB appear to be technically feasible, reproducible and safe. Sufficient MP tissue was obtained to identify myenteric neurons. These promising results are a step towards successful and safe implementation of these techniques into clinical practice for tissue diagnosis of muscle-based pathologies.

Introduction

Advancing our understanding of the pathophysiology of motility disorders, such as functional bowel disorders, chronic intestinal pseudo-obstruction and slow transit constipation, is hindered by our inability to non-invasively obtain muscularis propria (MP) to allow evaluation of multiple cell types, including myenteric neurons and interstitial cells of Cajal (ICC). Histologic analysis would allow for detection and differentiation of myopathic, neuropathic or ICC abnormalities, potentially providing essential information for diagnosis and therapy^{1,2,3,4}.

We have published a method to safely obtain endoscopic muscle biopsies from the human stomach⁵. Small bowel and colorectal muscle biopsies currently require a surgical approach, severely limiting the availability of diagnostic tissue. An effective, safe and efficient endoscopic technique capable of obtaining tissue that includes the intermuscular layer of the MP for evaluation of the enteric nervous system, immune cells and ICC may provide insights into the pathogenesis of these disorders. The aims of this study were to: 1) determine the technical feasibility, reproducibility and safety of performing a duodenal endoscopic muscle biopsy (dEMB) and rectal endoscopic muscle biopsy (rEMB) using a clip-assist technique; and 2) identify the muscle layers included in the resected specimens and the presence of myenteric neurons.

Materials and Methods

Experimental Design

This pre-clinical survival study in a pig model was approved by the Institutional Animal Care and Use Committee. Five pigs were studied. Each animal underwent a dEMB and rEMB procedure. dEMB was performed in the second part of the duodenum. Animals were survived for two weeks at which time an upper endoscopy was performed followed by necropsy. Resection sites were inspected for procedure related complications. The main outcome measures were efficacy and safety of the techniques, and histologic analysis of the resected tissue to determine which wall layers were included in the specimens and if MN were present. Data was recorded on the procedure, adverse events and clinical course of the animals. Data analysis was descriptive as this was a pilot feasibility study.

Animals

Five domestic 40 kg pigs were included. EMB were performed in each animal under general anesthesia and orotracheal intubation, following a 48 hour fast with water allowed ad libitum. A soap water enema was administered to each pig pre-procedure. Buprenorphine SR analgesic was administered once after anesthetic induction.

Procedure

a. dEMB—As the duodenal wall is relatively thin, we proceeded with a single resection clip-assist technique that would provide tissue of the entire wall. Porcine ductal anatomy is altered in that bile and pancreatic ducts exit into the duodenum separately. In general, the biliary orifice is located in the proximal duodenum/duodenal bulb and the pancreatic duct more distally. As such we targeted the lateral wall of the second part of the duodenum which clinically would also be the safer site for dEMB avoiding adjacent pancreas and mesenteric vessels. An over-the-scope clip (Padlock Clip – 11 mm; Aponos Medical, Kingston, NH) was fitted on a diagnostic upper endoscope (GIF-160; Olympus America, Center Valley, PA) and advanced to the post bulbar duodenum. This clip has an inner diameter of 11 mm and a cap depth of 10 mm. A tri-pronged tissue retraction device (OTSC Anchor, Ovesco Endoscopy, Tübingen, Germany) was passed through the working channel of the endoscope and deployed into the duodenal wall, which was then retracted and minimally suctioned into the cap of the clip. This device was preferentially chosen to ensure the entire gut wall or muscularis propria was adequately retracted into the cap. Once sufficient tissue was entrapped in the cap, the clip was deployed. The pseudopolyp of duodenum created was resected using a hot snare (AcuSnare; Cook Endoscopy, Bloomington, IN). Resection was done approximately 5 mm above the deployed clip to ensure ample tissue remained for a secure closure (Figures 1A and 1B). This approach resulted in a ‘no-hole’ dEMB. Effective closure of the biopsy site was confirmed during endoscopy by visible closure without disruption and sustained distension of the duodenal lumen with insufflation of air.

b. rEMB—Based on our previous experience with gastric endoscopic muscle biopsy (gEMB) we recognized the relative thickness of the rectal wall would likely not allow a single resection and proceeded with a double resection clip-assist technique to ensure MP was obtained in the resected tissues. Rectal resections were performed between 5 cms and 10 cms above the anal verge along the posterior wall. Clinically this would correspond to the mid-rectum, below the mid and above the distal valves of Houston, which would be the safer location for rEMB located below the peritoneal reflection and avoiding adjacent genitourinary structures. Endoscopic mucosal resection (EMR) using the band ligation approach (Duette; Cook Endoscopy, Bloomington, IN) was initially performed to expose the underlying MP. An over-the-scope clip (Padlock Clip – 11 mm; Aponos Medical, Kingston, NH) was fitted on a diagnostic upper endoscope (GIF-160; Olympus America, Center Valley, PA) and advanced to the exposed MP. A tri-pronged tissue retraction device (OTSC Anchor, Ovesco Endoscopy, Tübingen, Germany) was passed through the working channel of the endoscope and placed within the MP, which was retracted and minimally suctioned into the cap of the clip. Once adequate tissue was captured, the clip was deployed. The pseudopolyp of MP was resected using a hot snare (AcuSnare; Cook Endoscopy, Bloomington, IN) – Figures 2A and 2B. Similar to dEMB, effective closure of the biopsy

site was confirmed by secure closure without disruption and persistent distension of the rectal lumen with air insufflation.

Tissue Samples

Resected specimens were retrieved, pinned, measured and submitted for histology. A standard Hematoxylin and Eosin (H&E) staining protocol was used to determine which muscle layers were biopsied. Protein Gene Product 9.5 (PGP9.5) immunohistochemistry was used to visualize PGP9.5 positive neuronal fibers and myenteric neurons.

Post Procedure Care

Animals were placed on a liquid diet for 24 hours post- procedure, softened food on the second day, and on the third day regular feed was resumed as tolerated. Animals received ceftiofur 5mg/kg IM daily for 5 days and metronidazole 1 g bid for 5 days. After a two week survival period, repeat upper endoscopy was performed. Flexible sigmoidoscopy was not performed to avoid animal discomfort by administering an additional enema. Animals were chemically euthanized immediately after upper endoscopy which was followed by necropsy and inspection of the duodenal and rectal resection sites.

Results

dEMB and rEMB were successfully performed in all animals. There were no procedural complications. Mean procedure times for dEMB and rEMB were 23.7 ± 2.5 minutes and 13.25 ± 2.8 minutes, respectively. Mean length of resected full thickness duodenal wall was 13.25 ± 4.3 mm (Figure 3). The mean length of resected rectal MP was 12.5 ± 1.7 mm (Figure 4) and resected rectal mucosa and submucosa was 14.5 ± 3.1 mm. H&E stain showed MP with inner circular, outer longitudinal and intermuscular layers, including myenteric neurons in all the resected duodenal and rectal specimens (Figures 5A and 5B). Antibody to PGP 9.5 confirmed the presence of myenteric neurons in all samples (Figures 6A and 6B). Animals were survived for 2 weeks with an uneventful clinical course. Repeat upper endoscopy at 2 weeks showed well-healed scars without ulceration at the dEMB sites. Necropsy revealed no perforation, fluid collection, adjacent organ entrapment or abscess at either the duodenal or rectal resection sites. Mucosal views of the rEMB showed well healed scars while in two animals, single band adhesions were found at the dEMB sites (Figures 7A, 7B, 7C and 7D). At 2 weeks, retained clips were seen in all duodenal resection sites, and three rectal resection sites.

Discussion

This preclinical survival study evaluated the technical feasibility and safety of dEMB and rEMB using a 'no hole' (close then cut) technique avoiding overt perforation. Using this technique the entire MP that included circular and longitudinal muscle layers were obtained with sufficient tissue from the intermuscular layer to identify myenteric neurons. The significant advantage of this technique is defect closure prior to resection, thus preventing extraluminal leakage of any bowel contents or fluid. There were no procedure related complications. Animals had an uneventful clinical course with healing of all resection sites

and minimal adhesions at necropsy. Large tissue samples of the entire MP (10 mm) that included myenteric neurons were obtained from all animals. Procedure times were relatively brief (30 minutes) for both dEMB and rEMB.

All devices used in this study are commercially available in the United States. The star-shaped, over-the-scope clip was preferentially selected as the flat clip configuration facilitates easy snare placement and resection without entrapment of the clip prongs. This clip diameter of 11 mm allows for atraumatic peroral intubation, and a cap depth of 10 mm allows for ample tissue capture (Figures 8A and 8B). With this device the working channel of the endoscope can be used for additional accessories and suction of luminal contents as the trigger wire is located alongside the endoscope shaft. Furthermore, a submucosal injection prior to resection is not advocated. Based on our experience, this limits the adequacy of muscularis propria that can be subsequently retracted into the cap for resection.

The porcine J-shaped gastric anatomy restricted advancement of the endoscope to the post-bulbar duodenum but the above described dEMB technique could be easily applied to the jejunum using a dedicated enteroscope. Similarly, the porcine spiral colon configuration coupled by suboptimal bowel cleansing in animals restricted resection to the rectum. The longer procedure time for dEMB relates to negotiating and maintaining endoscope position within the porcine duodenum.

Although our study did not result in any adverse events, this remains a new procedure and appropriate caution must be exercised as with any novel technology. Potential complications include perforation (immediate or delayed), bleeding, stricture formation and adjacent organ entrapment. In order to minimize these risks we advocate use of minimal or no suction during tissue retraction into the cap to avoid entrapment of adjacent structures and resecting approximately 5 mm above the deployed clip to ensure ample tissue remains for a secure closure.

The concept and techniques of EMB for the purposes of tissue diagnosis have not been previously explored in the small bowel and rectum. The dEMB technique is similar to previously described clip-assist endoscopic full thickness resection (EFTR) of duodenal neoplastic lesions, however, we recommend using separate nomenclature (EMB versus EFTR) to clearly differentiate between diagnostic and therapeutic indications.

Our group recently published on effective over the scope clip-assisted EFTR of duodenal subepithelial lesions using a combination of clips and resection modalities⁶. Other small case series have shown a similar efficacy of clip-assist EFTR for duodenal lesions^{7,8,9}.

Furthermore, we published on a safe and effective gastric endoscopic muscle biopsy (gEMB) technique in patients with idiopathic gastroparesis⁵. rEMB like gEMB requires a double resection which is fundamental to acquisition of MP due to the relative thickness of the rectal wall. The versatility and applicability of the EMB technique to various parts of the gastrointestinal tract is appealing, as this allows for ease of use and effortless transition to clinical practice. A dedicated full thickness resection device (FTRD, Ovesco Endoscopy AG, Tubingen, Germany) is commercially available in Europe for colonic resections only, and is not currently FDA approved for use in the United States^{10,11}. However, in its present

iteration the larger device diameter would be challenging for safe peroral intubation for dEMB and may be suboptimal for rEMB which requires a double resection approach. Rectal EFTR has been described in a small series of rectal carcinoids using a single resection approach, however, only half the cases resulted in a full thickness resection highlighting the need for a double resection approach¹².

Intestinal MP biopsies could be potentially beneficial in patients with severe dysmotility of unknown etiology particularly those refractory to therapy, or in patients being considered for enteral or parenteral nutrition, and surgical intervention and in patients where an early therapeutic intervention is being considered such as steroid therapy and immunotherapy. Histologic findings can help identify abnormalities in the enteric nervous system^{13,14}. The role of inflammation and immune dysregulation in gastrointestinal diseases such as functional gastrointestinal disorders and pseudo-obstruction has not yet been adequately investigated^{4,15}. Reports have described histologic findings of inflammatory cell infiltration of the myenteric plexus or muscle, and their correlation to clinical and manometric findings^{16,17,18}. Such findings could potentially lead to targeted therapy, alleviating patient symptoms.

The ability to readily provide deep biopsies of the MP using safe, effective, minimally invasive, efficient and easy to perform endoscopic techniques represents a paradigm shift in endoscopic tissue diagnosis of gastrointestinal neuromuscular pathologies that may highlight new perspectives in the understanding and management of these disorders.

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A

B

Figure 1.

A. Endoscopic view of pseudopolyp of duodenal wall

B. Immediately post-dEMB (duodenal endoscopic muscle biopsy)

A

B

Figure 2.

A. Endoscopic mucosal resection (EMR) using the band ligation approach exposing the underlying muscularis propria (arrow) of the rectum

B. Endoscopic resection of pseudopolyp of rectal muscularis propria

Figure 3.
Resected duodenal wall

Figure 4.
Resected rectal muscularis propria

A

B **Figure 5.**

A. H&E stain (10X): Duodenal muscularis propria with inner circular muscle (CM), outer longitudinal muscle (LM) and intermuscular layer with myenteric plexus (MYP)

B. H&E stain (10X): Rectal muscularis propria with inner circular muscle (CM), outer longitudinal muscle (LM) and intermuscular layer with myenteric plexus (MYP)

A

B **Figure 6.**

A. Antibody to Protein Gene Product (PGP) 9.5 (10X): Duodenal muscularis propria with inner circular muscle (CM), outer longitudinal muscle (LM) and intermuscular layer with myenteric plexus (MYP)

B. Antibody to Protein Gene Product (PGP) 9.5 (10X): Rectal muscularis propria with inner circular muscle (CM), outer longitudinal muscle (LM) and intermuscular layer with myenteric plexus (MYP)

A

B

C

D

Figure 7.

- A. Necropsy: Mucosal view of duodenal resection site (black arrows) and clip border (white arrows)
- B. Necropsy: Serosa view of duodenal resection site with single band adhesion
- C. Necropsy: Mucosal view of rectal resection site (black arrows) and clip border (white arrows)
- D. Necropsy: Serosa view of rectal resection site

A

B **Figure 8.**

A. Star-shaped over-the-scope clip

B. Endoscopic view of clip (diameter 11 mm) fitted onto a diagnostic upper endoscope