Evaluation of endoscopic approaches for deep gastric-muscle–wall biopsies: what works?

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Background: A major barrier to furthering our understanding of the pathophysiology of neuromuscular GI diseases, including functional GI disorders, is the inability to obtain deep gastric-wall biopsy specimens that include both layers of the muscularis propria, which allows evaluation of specific cell types, including myenteric ganglia.

Objectives: The aims of this preclinical study were to (1) evaluate different endoscopic approaches for obtaining deep gastric-muscle–wall biopsy specimens and (2) determine if myenteric ganglia were present in the tissue samples.

Design and Interventions: This was a preclinical acute study by using a pig model. Multiple samples were obtained from 4 pigs. The endoscopic techniques evaluated were (1) EUS-guided tru-cut biopsy of the gastric wall, (2) jumbo biopsy of the post-EMR site, (3) jumbo biopsy of the gastrotomy margin, (4) serosal-side biopsy through a gastrotomy, and (5) double-EMR resection.

Main Outcome Measurements: Resected tissue was submitted for histology to determine which wall layers were included in the resected specimen. Hematoxylin and eosin staining was used to determine which muscle layers were biopsied, and an antibody to protein gene product 9.5 was used to determine if myenteric ganglia were present in the sample.

Results: Seventy-two tissue samples were obtained: EUS-guided tru-cut biopsy (n = 16), jumbo biopsy of the post-EMR site (n = 16), jumbo biopsy of the gastrotomy (n = 16), serosal-side biopsy (n = 16), and double-EMR resection (n = 8). Only the double-EMR resection tissues showed the presence of longitudinal muscle, indicating the presence of both muscle layers and the myenteric plexus. Immunofluorescence studies demonstrated the presence of myenteric ganglia only in the double-EMR tissues and in none of the other gastric samples. No adjacent organs were included in the resection.

Conclusions: The double-EMR technique was the only studied technique that resulted in a deep gastric-wall sample and provided sufficient tissue to evaluate both muscle layers and the intermuscular layer that contain myenteric ganglia. Further studies are needed to verify the efficacy and to assess the safety of this approach. (Gastrointest Endosc 2008;67:297-303.)

Functional dyspepsia is a common condition that remains essentially unexplained; abnormalities of gastric emptying, visceral sensation, and gastric accommodation have been reported, and the condition may be precipitated by acute GI infection.[1,2] Although the underlying mechanisms remain poorly defined, neuromuscular inflammation could conceivably be important in the pathogenesis, but this cannot be assessed by mucosal biopsy specimens. Similarly, idiopathic gastroparesis overlaps with functional dyspepsia and may occur after a presumed infectious insult.[3] Whether there are specific pathologic changes in the myenteric plexus in human gastroparesis remains unclear, but some experimental evidence strongly supports this concept.[4]

To further advance our understanding of these neuromuscular diseases, deep gastric-wall biopsy specimens that include the muscularis propria are needed to

Abbreviations: EMRC, cap-assisted EMR; NDS, normal donkey serum; NOTES, natural orifice transluminal endoscopic surgery; PBS, phosphate buffered saline solution; PGP9.5, protein gene product 9.5.

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determine if inflammation or abnormalities of neuromuscular structures, such as the interstitial cells of Cajal, may explain the observed pathophysiologic disturbances. Current methodology to obtain such a biopsy specimen requires surgery, which severely limits the availability of tissue. Mucosal endoscopic biopsy specimens are insufficient; a deep gastric-wall biopsy specimen is required to provide tissue for histologic analysis of the muscle layers of the stomach, as well as the plexi present between the circular and longitudinal muscle layers. The ability to obtain such tissue would permit the application of specialized staining techniques to detect and characterize myenteric ganglia, as well as the interstitial cells of Cajal and immune cells.

The aims of this preclinical study were to (1) evaluate different endoscopic approaches for obtaining deep gastric-muscle–wall biopsy specimens that include the muscularis propria (oblique, circular, and longitudinal muscle layers) and to (2) determine if myenteric ganglia were present in the tissue samples.

Capsule Summary

What is already known on this topic

- The inability to obtain deep gastric-wall biopsy specimens that include both layers of the muscularis propria prevents a full understanding of the pathophysiology of neuromuscular GI diseases.

What this study adds to our knowledge

- In a comparison of 5 endoscopic approaches to deep gastric-wall biopsies, only tissue obtained by double EMR indicated the presence of both muscle layers and the myenteric plexus; likewise, immunofluorescence studies demonstrated myenteric ganglia only in the double-EMR tissues.

MATERIALS AND METHODS

Experimental design

This study was approved by the institutional animal care and use committee. Four pigs were approved to be studied, with multiple samples obtained from the gastric body and the antrum of each pig. The animals underwent the following endoscopic techniques:

1. EUS guided tru-cut biopsy of the gastric wall
2. Jumbo biopsy of post-EMR site
3. Jumbo biopsy of a gastrotomy margin
4. Serosal-side retroflexed biopsy through a gastrotomy
5. Double-EMR resection

This was an acute study with animals euthanized immediately after the procedure. At necropsy, full-thickness tissue resections were obtained from the gastric antrum and the body to serve as controls for the presence of myenteric ganglia in porcine muscularis propria. The resection sites were also inspected for procedure-related complications.

The main study outcome measurements were histologic analysis of the resected tissue to determine which wall layers were included in the resected specimen and if myenteric ganglia were present in the sample. Data analyses were descriptive, because this was a pilot feasibility study.

Animals

Four domestic, 30-kg pigs were studied. An endoscopy was performed in each animal, with the animal under general anesthesia and orotracheal intubation, after a 48-hour fast. Animals were chemically euthanized immediately after the procedure, and a necropsy was performed.

Procedures

EUS-guided tru-cut biopsy of the gastric wall. EUS-guided tru-cut biopsy of the gastric wall is seen in Figure 1A. By using a 19-gauge Tru-cut biopsy needle (Quick-Core; Cook Endoscopy, Winston-Salem, NC), a gastric-wall biopsy was performed with a linear echoendoscope (Aloka–GIF-UC140P-AL5; Olympus America, Center Valley, Pa). The Tru-cut biopsy needle was directed as tangentially as possible into the gastric muscularis propria in an effort to position the tissue-specimen tray of the needle within the muscularis propria, maximizing tissue acquisition. Biopsy specimens were obtained from the gastric body and the antrum.

Jumbo biopsy of post-EMR site. A jumbo biopsy of the post-EMR site is presented in Figure 1B. Cap-assisted EMR (EMRC) was initially performed without a protective submucosal cushion. The resected tissue was extracted, and the exposed muscle layer was biopsied by using a jumbo biopsy forceps (Olympus America). Biopsy specimens were obtained by using this technique from the gastric body and the antrum.

Jumbo biopsy of the gastrotomy. Jumbo biopsy of the gastrotomy is presented in Figure 1C. By using a fistulotome (Olympus America), a gastrotomy was made through the anterior wall of the gastric body. A guidewire was passed through this opening, a balloon dilator (CRE; Microvasive Endoscopy, Boston Scientific Corp, Natick, Mass) was passed over the wire, and the fistula was dilated to an 8-mm gastrotomy. Biopsy specimens were then obtained from the margin of the gastrotomy by using a jumbo biopsy forceps.

Serosal-side biopsy through a gastrotomy. A serosal-side biopsy through a gastrotomy is shown in Figure 1D.

This technique was performed after the ''jumbo biopsy of the gastrotomy.'' The existent natural orifice transluminal endoscopic surgery (NOTES) type gastrotomy was further dilated to 15 mm by using a balloon dilator, and the endoscope was advanced through the opening into the peritoneal cavity. Biopsy specimens were obtained by