Comparison of intestinal leak pressure between cadaveric canine and commercial synthetic intestinal tissue that did and did not undergo enterotomy

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OBJECTIVE

To compare initial leak pressure (ILP) between cadaveric canine and synthetic small intestinal segments that did and did not undergo enterotomy.

SAMPLE

Eight 8-cm grossly normal jejunal segments from 1 canine cadaver and eight 8-cm synthetic small intestinal segments.

PROCEDURES

Intestinal segments were randomly assigned to undergo enterotomy (6 cadaveric and 6 synthetic segments) or serve as untreated controls (2 cadaveric and 2 synthetic segments). For segments designated for enterotomy, a 2-cm full-thickness incision was created along the antimesenteric border. The incision was closed in a single layer with 4-0 suture in a simple continuous pattern. Leak testing was performed with intestinal segments occluded at both ends and infused with dilute dye solution (999 mL/h) until the solution was observed leaking from the suture line or serosal tearing occurred. Intraluminal pressure was continuously monitored. The ILP at construct failure was compared between cadaveric and synthetic control segments and between cadaveric and synthetic enterotomy segments.

RESULTS

Mean ± SD ILP did not differ significantly between cadaveric (345.11 ± 2.15 mm Hg) and synthetic (329.04 ± 24.69 mm Hg) control segments but was significantly greater for cadaveric enterotomy segments (60.77 ± 15.81 mm Hg), compared with synthetic enterotomy segments (15.03 ± 6.41 mm Hg).

CONCLUSIONS AND CLINICAL RELEVANCE

Leak testing should not be used to assess the accuracy or security of enterotomy suture lines in synthetic intestinal tissue. Synthetic intestinal tissue is best used for students to gain confidence and proficiency in performing enterotomies before performing the procedure on live animals. (*Am J Vet Res* 2020;81:827–831)

The use of live animals for terminal surgical procedures in veterinary training programs has been decreasing in recent years owing to the ongoing trend toward reduction, refinement, and replacement of live animals for teaching and research purposes.[1–4] Consequently, the use of cadavers and synthetic models for training purposes has been increasing.[3–5] In both veterinary and human medicine, many commercial synthetic intestinal models have been developed to replace live animals and patients or cadavers in medical device studies, surgical simulations, and clinical training programs.[6–11]

Veterinary curricula and continuing education programs are increasingly embracing modern training modalities, such as simulations and the use of synthetic surgical models, because they improve student confidence and proficiency in a procedure before advancing to live animal experiences. These modalities also reduce the costs associated with live animal surgeries and decrease the number of live animals and cadavers required for students to gain proficiency, which is important because the acquisition of cadavers has become increasingly difficult at many institutions.[12–14] Students enrolled in curricula that use simulated or synthetic models have more confidence and perform better when they transition to live animal training, compared with students enrolled in curricula that do not use such models.[12,13] Additionally, synthetic tissue can be easily stored and more readily obtained for use in mass teaching exercises, compared with obtaining and preserving cadaveric tissue. Despite the many benefits associated with the use of synthetic models for surgical training, many factors unique to cadaveric tissue or live animals, such as tissue texture, hemorrhage, and mucosal eversion, are difficult or impossible to replicate with synthetic models.

ABBREVIATIONS

ILP Initial leak pressure

In veterinary practice, surgical procedures of the gastrointestinal tract, such as enterotomy and intestinal resection and anastomosis, are commonly performed to alleviate obstructions and collect biopsy specimens. However, veterinary students have limited opportunities to perform or become proficient in gastrointestinal surgery on live animals during their training programs. Synthetic tissue represents an alternative training model and provides students the opportunity to practice surgical procedures, which is important given the difficulty and cost associated with obtaining live animals and cadavers for training purposes at many institutions. During enterotomy procedures on live animals, particularly those performed by novice surgeons, an intraoperative leak test is recommended to assess enteric suture line security and identify imperfections in closure technique with the goal of preventing postoperative leakage.[15–18] It is unknown whether leak testing of enterotomy sites in synthetic intestinal tissue is as accurate as leak testing of enterotomy sites in canine cadaveric intestinal tissue for evaluation of suture line integrity. Additionally, to our knowledge, the intraluminal pressures capable of being sustained before and after enterotomy in synthetic intestinal tissue, compared with those in canine cadaveric tissues, have not been investigated.

The objective of the study reported here was to compare the intraluminal pressure at the time leakage was first observed during leak testing (ie, ILP) between synthetic and canine cadaveric intestinal segments that did and did not undergo a simple enterotomy. Our hypotheses were that the ILP would not differ significantly between synthetic and cadaveric intestinal segments that did not undergo enterotomy (ie, control segments), and that the ILP for cadaveric intestinal segments that underwent enterotomy would be greater than that for synthetic intestinal segments that underwent enterotomy.

Materials and Methods

Specimens

The study was reviewed and approved by the University of Florida Institutional Animal Care and Use Committee (No. 201810281). Cadaveric intestinal specimens were harvested from 1 middle-aged sexually intact male mixed-breed dog that weighed between 20 and 25 kg and was euthanized with an IV infusion of pentobarbital–phenytoin sodium for reasons unrelated to the study. An approximately 64-cm segment of jejunum was harvested from the dog within 2 hours after euthanasia; no gross abnormalities were observed in the gastrointestinal tract during intestine harvest. The harvested jejunal segment was immediately submerged in sterile saline (0.9% NaCl) solution and stored at 4°C for < 24 hours prior to experimental testing. Immediately prior to experimental testing, the segment was removed from the saline solution and cut into eight 8-cm sections. The luminal contents were gently milked from each section, then each section was flushed with sterile saline solution.

Eight 8-cm segments of commercially available synthetic intestinal tissue[a] comprised of water, salt, and fiber were used for the study. The segments were stored in an appropriate solution in accordance with the manufacturer's tissue care and storage guidelines.

Two segments of cadaveric intestine and 2 segments of synthetic intestine were randomly selected by means of a random number generator to serve as untreated control segments (ie, did not undergo enterotomy). The remaining 6 cadaveric intestine and 6 synthetic intestine segments underwent a simple enterotomy.

Surgical technique

All enterotomies were performed by 1 board-certified veterinary surgeon (PJR) in accordance with a standardized technique. For each 8-cm intestinal segment (both cadaveric and synthetic), a No. 11 scalpel blade was used to create a 2-cm full-thickness incision along the antimesenteric border in the middle of the segment. The incision was closed in a single layer by use of 4-0 glycomer 631 suture with a swaged CV-23 half-circle, 17-mm tapered needle in a simple continuous pattern. The suture bites were placed 2 to 3 mm from each tissue edge and 2 to 3 mm apart. The body of the needle was used to replace any everted mucosa into the intestinal lumen as tension was applied to each suture loop. Tension was placed on the suture after each pass through the tissue to maintain constant, noncrushing pressure on the tissue. The suture pattern was secured with 4 complete square knots.

Leak testing

All intestinal segments underwent leak testing within 6 hours after completion of the enterotomy procedures. Both ends of each intestinal segment were occluded with Rochester-Carmalt forceps. Two 18-gauge, 1.5-inch catheters were inserted through the intestinal wall into the occluded portion of the lumen on the antimesenteric border adjacent to the forceps. The catheterized segment was suspended over a clear trough to facilitate monitoring for leakage. Both catheters were connected to a pressure transducer, transducer amplifier, and pressure monitoring system via IV fluid tubing **(Figure 1)**. One of the catheters was also connected to a syringe pump with a 3-way stopcock and another set of IV fluid tubing. Prior to testing, all IV fluid tubing was purged of air and filled with saline solution. The transducers were calibrated with a manometer before each test and connected to a data acquisition system. Methylene blue was added to a 1-L bag of physiologic saline solution (dilution, 1:500) to aid visual observation of leakage. The dilute methylene blue solution was infused into the occluded intestinal segment via the syringe pump at a rate of 999 mL/h. The pressure monitoring equipment continuously recorded and displayed the intralumi-

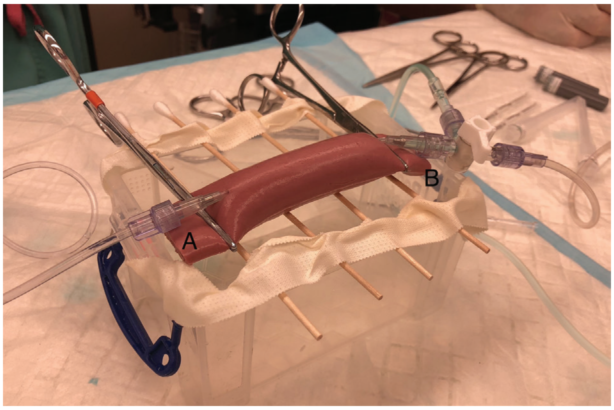

Figure 1—Photograph depicting the leak testing construct used in a study conducted to compare the ILP between cadaveric canine and synthetic small intestinal segments that did and did not undergo enterotomy. In this photograph, both ends of an 8-cm segment of untreated (control) synthetic small intestinal tissue were occluded with Rochester-Carmalt forceps. Two 18-gauge, 1.5-inch catheters (A and B) were inserted through the segment wall into the occluded portion of the lumen along the antimesenteric border adjacent to the forceps. The catheterized segment was suspended over a clear trough to facilitate monitoring for leakage. Catheter A was connected to a transducer, and catheter B was connected to a transducer and syringe pump (not shown) via IV fluid tubing, which was purged of air and filled with saline (0.9% NaCl) solution prior to testing. The transducers were calibrated with a manometer before each test and connected to a data acquisition system. Methylene blue was added to a 1-L bag of physiologic saline solution (dilution, 1:500) to aid visual observation of leakage. The dilute methylene blue solution was infused into the occluded intestinal segment via the syringe pump at a rate of 999 mL/h. The pressure monitoring equipment continuously recorded and displayed the intraluminal pressure. The segment was tested until failure, which was defined as the observation of serosal tearing or leakage of the methylene blue solution around the infusion catheter or pressure transducer, or until the maximum pressure for the sensor system (350 mm Hg) was reached.

nal pressure. All specimens were tested until failure, which was defined as the observation of serosal tearing or leakage of the methylene blue solution around the infusion catheter, pressure transducer (control specimens), or any point along the enterotomy site, or until the maximum pressure for the sensor system (350 mm Hg) was reached. All specimens were observed for leakage by 1 investigator (PJR), and the pressure at the time of failure (ILP) was recorded by another investigator (MJF). For any segment that did not fail before the maximum pressure for the sensor system was achieved, the recorded ILP was 350 mm Hg.

Statistical analysis

The 4 treatment groups were defined as follows: cadaveric control, cadaveric enterotomy, synthetic control, and synthetic enterotomy. The ILP data distribution for each group was assessed for normality by visual examination of histograms and the Shapiro-Wilk test. All data were normally distributed, and results were summarized as the mean ± SD. *t* Tests were used to compare the ILP between the cadaveric and synthetic control groups and between the cadaveric and synthetic enterotomy groups. Values of $P \leq 0.05$ were considered significant. All analyses were performed with commercially available statistical software.[b]

Results

The mean ± SD ILP for the cadaveric control group (345.11 ± 2.15 mm Hg) did not differ significantly ($P = 0.50$) from that for the synthetic control group (329.04 ± 24.69 mm Hg), although there was a 4.7% difference between the 2 means. The mean ± SD ILP for the cadaveric enterotomy group (60.77 ± 15.81 mm Hg) was significantly ($P = 0.002$) greater than that for the synthetic enterotomy group (15.03 ± 6.41 mm Hg), and there was a 75.3% difference between the 2 means.

Discussion

Results of the present study supported our hypotheses that the mean ILP would not differ significantly between cadaveric and synthetic control segments, and that the mean ILP for cadaveric segments that underwent enterotomy would be significantly greater than that for synthetic segments that underwent enterotomy. The maximum pressure that could be quantified by the pressure transducer used for leak testing of the intestinal segments of the present study was 350 mm Hg. The mean ILP for both cadaveric and synthetic control segments approached that maximum pressure limit, and the ILP could not be exactly quantified for some control segments.

In another study,[19] cadaveric canine jejunal segments that were infused with dyed saline solution at a rate of 999 mL/h (the same infusion rate used during leak testing for the intestinal segments of the present study) following enterotomy had a mean ± SD bursting pressure of 93.63 ± 24.10 mm Hg. That bursting pressure was much higher than the mean ILP measured for the cadaveric and synthetic segments that underwent an enterotomy in the present study. We believed that the ILP was a more clinically relevant measure than bursting pressure; therefore, we did not measure bursting pressure for the intestinal segments of the present study. In unanesthetized healthy dogs, the intraluminal pressure in the jejunum during peristalsis is estimated to be 15 to 25 mm Hg.[20] Thus, the mean ± SD ILP achieved for the cadaveric (345.11 ± 2.15 mm Hg) and synthetic (329.04 ± 24.69 mm Hg) control intestinal segments of the present study were supraphysiologic and exceeded the mean ILP achieved for control intestinal segments of other studies.[21,22]

In the present study, the mean ± SD ILP for the cadaveric enterotomy group (60.77 ± 15.81 mm Hg) was significantly greater than that for the synthetic enterotomy group (15.03 ± 6.41 mm Hg). In fact, the mean ILP for the synthetic enterotomy group was similar to the estimated lower limit for jejunal intraluminal pressure during peristalsis (15 to 25 mm Hg) in healthy dogs.[20] For all cadaveric and synthetic intestinal segments that underwent enterotomy in the present study, everted mucosa was inverted into the intestinal lumen, as is recommended for clinical patients, and the enterotomy incisions were closed with a single layer of sutures placed in a simple continuous pattern as in other studies.[21,23] Cadaveric intestinal tissue is softer, less elastic, and more compressible than synthetic intestinal tissue.[24] We suspect that the inherent compliant nature of the cadaveric tissue resulted in a more immediate and tighter seal at the sutured enterotomy site than did the less compliant synthetic tissue, which contributed to the large difference in mean ILP between the 2 groups. Consequently, we do not recommend leak testing of enterotomy sites in synthetic intestinal tissue as a method to evaluate or identify imperfections in enterotomy closure techniques.

The mean ILP for the synthetic (15.03 mm Hg) and cadaveric (60.77 mm Hg) enterotomy segments of the present study varied from the mean ILP reported for cadaveric canine intestinal segments that underwent enterotomy (range, 14.4 to 34.0 mm Hg) in other studies.[21,22,25] The large variation in the mean ILP for intestinal segments that did and did not undergo experimental enterotomy in the veterinary literature likely reflects differences in the leak testing protocols used among studies. The leak testing protocol used for the present study was selected because of its ease of construction, and it was similar to that used to evaluate the ILP in cadaveric porcine jejunal segments of another study.[26] Also, the mean ILPs calculated for the control intestinal segments of this study were consistent with those calculated during pilot testing of the protocol for this study and for the porcine jejunal segments of that other study.[26] The leak testing constructs used in other studies[21,22] were more complex than the construct used in the present study and difficult to replicate. Results of another study[27] indicate that the ILP achieved at enterotomy sites during ex vivo leak testing is greater than the intraluminal pressure expected at the enterotomy sites of clinical patients. Thus, the ILPs determined during testing of ex vivo intestinal segments may not be directly comparable to ILPs in vivo. Differences in the intraluminal volume and method used to occlude intestinal segments during leak testing can affect the ILP.[18] Variation in the infusion rate of saline solution during leak testing can also affect the ILP. High infusion rates may cause constructs to fail quicker at lower pressures than lower infusion rates owing to differences in surface tension.

The present study had multiple limitations, such as its ex vivo nature and small sample size. We limited the number of intestinal segments per enterotomy group to 6 to be consistent with previous studies[21,22] and owing to the availability of synthetic intestinal tissue. Although intestinal leak testing is routinely performed in experimental settings, it does not accurately represent a typical physiologic state because in vivo jejunal segments are rarely distended to the same magnitude as ex vivo segments during leak testing. Thus, the leak testing protocol performed in the present study represented only a crude indicator of enterotomy strength and integrity. All cadaveric jejunal specimens evaluated in the present study were obtained from 1 dog, and even though they appeared grossly normal, they might not have been representative of healthy jejunal specimens in the general population of dogs. There was likely some variation in the suturing technique used to close the enterotomy sites, although we tried to minimize that variation by having the same investigator perform all enterotomies. All intestinal segments of this study were tested to failure by gradual constant infusion of dilute methylene blue solution; they did not undergo cyclic infusion or anything else to simulate peristalsis, which would have been more clinically relevant. Finally, it is important to note that the Laplace law states there is an inverse relationship between intraluminal pressure and radius.[28] Although all cadaveric jejunal segments tested in this study were from the same dog and appeared to be of similar size, the intraluminal radius was not measured or compared between cadaveric and synthetic specimens. Thus, some of the differences in ILP observed between the treatment groups might have been attributable to differences in the intraluminal radius between cadaveric and synthetic intestinal segments.

Currently, synthetic biomaterials represent oversimplified imitations of natural extracellular matrices and lack essential natural temporal and spatial complexity.[24] Investigators of a study[29] conducted to assess the use of various models for training veterinary students to perform enterotomies concluded that cadaveric intestine more closely approximated in vivo intestine than did synthetic tissue models when variables such as tissue handling, needle passage, and mucosal eversion were evaluated. Conversely, investigators of another study[30] concluded that synthetic intestinal tissue used in conjunction with a machine to mimic peristalsis in human patients worked as well as porcine intestinal tissue for student training and mitigated the need for cadaveric tissue. Use of surgical models for training improves student confidence and performance when they transition to performing procedures on live patients.[12,13] A considerable amount of evidence supports the effectiveness of surgical simulation models for training in the health professions, particularly in human medicine.[7-11]

In the present study, the ILP was similar between untreated (control) cadaveric and synthetic intestinal segments, but for segments that underwent enterotomy, the ILP for cadaveric segments was significantly greater than that for synthetic segments. Therefore, we concluded that when synthetic intestinal tissue is used for enterotomy training, leak testing should not be used to assess the accuracy or security of the enteric suture line. Instead, such models should be used to allow students the opportunity to gain confidence and proficiency in performing the enterotomy procedure before performing it on live animals.

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Drs. Case and Kim are patent holders (University of Florida No. 1607) of SynDaver Canine, the company that produced the synthetic intestinal tissue used in this study.

Footnotes

a. SynDaver Labs, Tampa, Fla.
b. JMP, version 14, SAS Institute Inc, Cary, NC.

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