Safety and effectiveness of mechanical versus hand suturing of intestinal anastomoses in an animal model of peritonitis

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Abstract. Mechanical stapling for colorectal anastomosis is popular, but the safety of its use for anastomosis in peritonitis is unclear. We evaluated the safety and effectiveness of mechanically stapled vs. hand-sutured anastomosis by comparing wound healing in an animal model of bacterial peritonitis. Male Sprague-Dawley (n=48) rats underwent cecal ligation and puncture. After 24 h, rats were divided into two groups: the stapler group (cecal resection with mechanical stapler, n=24) and the hand-sutured group (cecal resection and stump closure with surgical absorbable suture, n=24). Anastomotic segments were excised and as indicators of wound healing, anastomotic bursting pressure (ABP) and tissue hydroxyproline concentration were determined over time. After harvesting, anastomotic segments were analyzed by quantitative real-time polymerase chain reaction (PCR) to determine relative expression of transforming growth factor-β_1 (TGF-β_1) and vascular endothelial growth factor (VEGF) normalized to that of a constitutive gene. The operative time was significantly shorter in the stapler vs. the hand-sutured group. Both groups showed progressive increases in ABP over the postoperative period. ABP was significantly higher in the stapler vs. the hand-sutured group on postoperative days (PODs) 0 and 3. Tissue hydroxyproline concentration increased from POD 7 in both groups, but between-group difference was not significant. Both groups showed progressive increases in TGF-β_1 and VEGF expression during the 7-day postoperative period. On POD 5, TGF-β_1 gene expression was higher in the stapler vs. the hand-sutured group. VEGF gene expression was identical in both groups. In conclusion, anastomosis by stapler is safer and more effective than that by hand suturing in bacterial peritonitis, since it requires less operating time and creates stronger anastomoses in the early postoperative period.

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Introduction

Anastomotic dehiscence following gastrointestinal surgery, particularly colorectal surgery, is a significant cause of morbidity and mortality, and leakage from colonic anastomosis is a major concern for surgeons. The incidence of reported anastomotic leakage varies between 10 and 13% (1,2). Presently, the use of mechanical staplers for colorectal anastomoses has been increasing and is becoming widely accepted in Western countries due to the short procedure time and reliability afforded by this technique. It has reduced the operation time, has facilitated the performance of gastrointestinal anastomoses at sites with a poor field of view and has decreased the likelihood of suture failure (3,4). Several prospective randomized controlled studies have compared hand suturing with mechanical stapling in patients undergoing total gastrectomy and low anterior colorectal resection (5-7). However, hand-sutured anastomoses are still popular due to economic conditions in developing countries, and few experimental studies have yet been reported comparing wound healing of stapled anastomosis with hand-sutured anastomosis, particularly in conditions of peritonitis.

Many factors contribute to wound healing and the integrity of an anastomosis, such as blood supply, tension of the anastomosis, bowel preparation, patient condition and inflammation (8). In addition, peptide growth factors (PGFs) play a significant role in wound healing. These molecules have been shown to mediate the stages of wound healing, including neovascularization and synthesis, deposition and maturation of collagen. Among these PGFs, transforming growth factor-β_1 (TGF-β_1) has been found to play the most important role in anastomotic wound healing, including inflammation, fibroplasia and deposition of collagen (9,10). Similarly, the most important cytokine implicated in neovascularization processes is vascular endothelial growth factor (VEGF) (11).

The aim of the present study was to evaluate the safety and effectiveness of mechanical stapled anastomosis vs. hand-sutured anastomosis in an animal model of peritonitis by comparison of wound healing between these two styles of anastomoses.

Materials and methods

Study design. Adult male Sprague-Dawley rats were allowed to acclimate to laboratory conditions for 1 week prior to

experimental use. Animals were housed at 21˚C with a 12-h day-night cycle. They had free access to water and standard laboratory chow. The study protocol was approved by the Animal Ethics Review Committee of the Oita University, Faculty of Medicine (Oita, Japan).

Surgical procedure. In 48 rats weighing 250-300 g each, bacterial peritonitis was induced using a cecal ligation and puncture (CLP) model, with minor modification (12). Rats were fasted for 24 h and anesthetized with ether. The abdomen was shaved and disinfected with 70% alcohol and a 2-cm midline incision was made. The cecum was dissected without damaging its vascular supply and was filled with feces by milking stool back from the ascending colon. Thereafter, the cecum was ligated 5 mm below the ileocecal valve with 3-0 silk suture and punctured twice with an 18-gauge needle at the antimesenteric site. The abdominal wall was closed in two layers. Immediately after the operation, rats received saline subcutaneously (5 ml/100 g body weight) and were placed in cages. After 24 h, the abdomen was reopened and cecal resection was performed. Rats were randomized into two groups (n=24 each): the stapler group, in which the cecum was resected just below the ileocecal valve with a 6-row linear stapler (Endo-GIA Universal; Covidien, Mansfield, MA, USA) and the hand-sutured group, in which the cecum was resected just above the ligation followed by closure of the stump with four interrupted two-row Albert-Lembert suture using 6-0 polydioxanone sutures (PDS II; Ethicon, Somerville, NJ, USA). The abdomen was closed in two layers. The operative time was recorded in all surgical procedures. Rats were maintained on standard laboratory chow and water *ad libitum* after surgery. They were sacrificed on postoperative day (POD) 0 (immediately after surgery) and on PODs 3, 5 and 7 after surgery under full inhalant anesthesia with ether.

The abdominal wall was disinfected with 70% alcohol and reopened. For measurement of bursting pressure, the anastomoses were carefully resected by transecting at the ileum and the ascending colon so as not to injure the intestinal wall. After determination of the bursting pressure, colonic tissue samples consisting of a 0.5-cm segment of perianastomotic tissue were obtained 0.5 cm proximal to the anastomotic line. The colonic tissue samples were snap-frozen in liquid nitrogen and then stored at -80˚C for later use.

Anastomotic bursting pressure. The anastomotic bursting pressure (ABP) was measured on postoperative days (PODs) 0, 3, 5 and 7. Fecal material was removed from the ileal and colonic segments. A tube was inserted into the cecum from the ileal segment. Then, two ligations were made at the ascending colon and the ileum. The tube was connected to an infusion pump and a pressure transducer was linked to a recorder. Pressure was measured during infusion of normal saline solution through the tube at a constant rate of 1 ml/min. The pressure recorded immediately before abrupt loss of pressure was considered the ABP.

Total-RNA isolation. Total-RNA was isolated from frozen intestinal samples with an EZ1 RNA Tissue Mini kit (Qiagen, Tokyo, Japan) following the manufacturer's protocol. Complementary DNA (cDNA) was synthesized as described previously (13). The RNA was resuspended in DEPC-treated water and stored at -80˚C until the reverse transcription step. The concentration of the RNA was determined with a spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Total-RNA (1.0 μg) was reverse-transcribed at 37˚C for 60 min in the presence of a 25-μl reaction containing 80 pmol random primer (Takara Bio, Inc., Shiga, Japan) and 200 U Moloney murine leukemia virus reverse transcriptase (M-MLV Reverse Transcriptase; Invitrogen, Tokyo, Japan) according to the manufacturer's protocol. The cDNA was used as a template for subsequent real-time polymerase chain reaction (PCR).

Real-time PCR. Quantitative real-time PCR was carried out with a Light-Cycler System (Roche Diagnostics, Lewes, East Sussex, UK). Primer sets for TGF-β_1 and VEGF were purchased from Search-LC (Heidelberg, Germany) (rat TGF-β_1 set no. 410636 and rat VEGF set no. 4410874). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH primer set; Search-LC) was amplified according to the manufacturer's protocol as an internal control to allow quantitation of the TGF-β_1 and VEGF amplification products. A fresh standard dilution series was prepared. The PCR mix, which contained 9.4 μl PCR-grade water, 1 μg of each TGF-β_1- and VEGF-specific primers (10 μM), 1.6 μl of 25 mM $MgCl_2$ and 2 μl LightCycler FastStart DNA Master SYBR Green I, was added to a 1.5-ml light-protected LightCycler capillary and 5 μl cDNA template (diluted 10X) were added. The PCR mix (15 μl) was pipetted into 4 precooled LightCycler capillary tubes, and 5 μl undiluted and 5 μl freshly diluted standard were then added to each capillary. Each capillary was sealed with a stopper and centrifuged at 700 x g for 15 sec. The capillaries were placed into the rotor of the LightCycler and the samples were amplified. PCR cycles were monitored continuously with SYBR Green I dye. After amplification, melting curve analysis permitted accurate identification of the PCR amplicons. Data were analyzed with LightCycler analysis software (Roche Diagnostics) and a standard curve that correlated cycle number with the amount of product formed was plotted for each sequence of interest. TGF-β_1 and VEGF expression levels were then normalized to that of GAPDH.

Hydroxyproline assay. Hydroxyproline concentrations in the anastomotic segment on the operative day and PODs 3 and 7 were measured as an indicator of collagen accumulation (14). This assay was performed on tissue samples frozen in liquid nitrogen and kept at -80˚C in a deep freezer. After measuring the wet tissue weight, the samples were hydrolyzed by adding 6 N HCl at 110˚C for 24 h in sealed test tubes. Hydrolysates were transferred to flasks and dried overnight in desiccators to remove the hydrochloric acid by evaporation. After dissolving the hydrolysates in 3 ml of 0.02 N HCl, 0.1 ml of the solution was extracted and added to 0.9 ml of 0.02 N HCl. Final solutions were applied in 50-μl aliquots to the amino acid analyzer to determine the hydroxyproline concentration.

Statistical analysis. All data are expressed as means ± standard deviation. Statistical analysis was performed by means of the Mann-Whitney U test. A value of $P<0.05$ was considered to indicate statistical significance. The Dr SPSS II for Windows 11.0.1J program (IBM Japan, Tokyo, Japan) was used for all statistical analyses.

Table I. Operative times in the stapler and hand-sutured groups.

	Stapler group	Hand-sutured group	P-value
Operative time[a]	9.1±2.8	15.9±2.4	<0.001

[a]in minutes. Values are expressed as means ± SD.

Results

Operative findings and ABP. All animals tolerated the surgical intervention well and no animal died prematurely during the study. The operative time was significantly shorter in the stapler group than in the hand-sutured group (9.1±2.8 vs. 15.9±2.4 min, P<0.001, respectively) (Table I). Both groups showed progressive increases in ABP over the postoperative period (Fig. 1). The stapler group had a significantly higher ABP than the hand-sutured group on PODs 0 and 3, but the difference was not statistically significant on PODs 5 and 7.

Local temporal gene expression of the PGFs. Gene expression of TGF-β_1 and VEGF on the days studied during the first 7 PODs is shown in Fig. 2. Both groups showed progressive increases in the expression of TGF-β_1 and VEGF over the 7-day postoperative period. On POD 5, the stapler group showed a higher level of gene expression of TGF-β_1 than that of the hand-sutured group (Fig. 2A). Gene expression of VEGF was identical in both groups (Fig. 2B).

Hydroxyproline assay. Tissue hydroxyproline concentration around the anastomotic segments on PODs 0, 3 and 7 is shown in Fig. 3. Although the concentration of hydroxyproline began to increase from POD 7 in both groups, there was no significant difference between the two groups.

Discussion

Mechanically stapled anastomoses are clinically common in Western countries. However, to our knowledge, there are no reports comparing wound healing of intestinal mechanically stapled anastomosis with that of hand-sutured anastomosis in an animal model of peritonitis. The model of CLP, as reported by Wichterman *et al* (12), produces an early and late phase of sepsis. In the first 16 h (early phase), animals show typical hyperdynamic features, whereas those in the late phase of sepsis show hypodynamic features as in peritonitis. In our experiment, the anastomosis was constructed during the late phase of sepsis, but the ongoing source of infection (ligated perforated cecum) was removed to allow the animals to recover. To compare the integrity and healing process of the anastomoses, we determined the bursting pressure of the anastomoses, concentration of hydroxyproline and local temporal expression of TGF-β_1 and VEGF. Our present results showed that the anastomosis made by stapler is safer and more effective than that made by hand suturing in an animal model of bacterial peritonitis, because of the stronger anastomosis created in the early postoperative period and the shorter operating time afforded by the stapler.

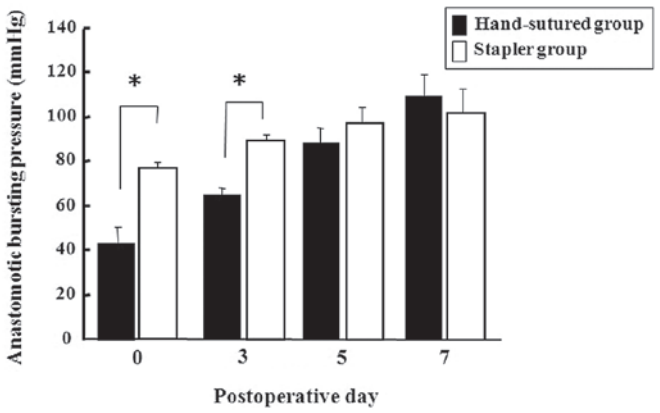

Figure 1. Median anastomotic bursting pressure in the stapler and hand-sutured groups on postoperative days 0, 3, 5 and 7. Error bars represent the standard deviation of the mean. *P<0.05.

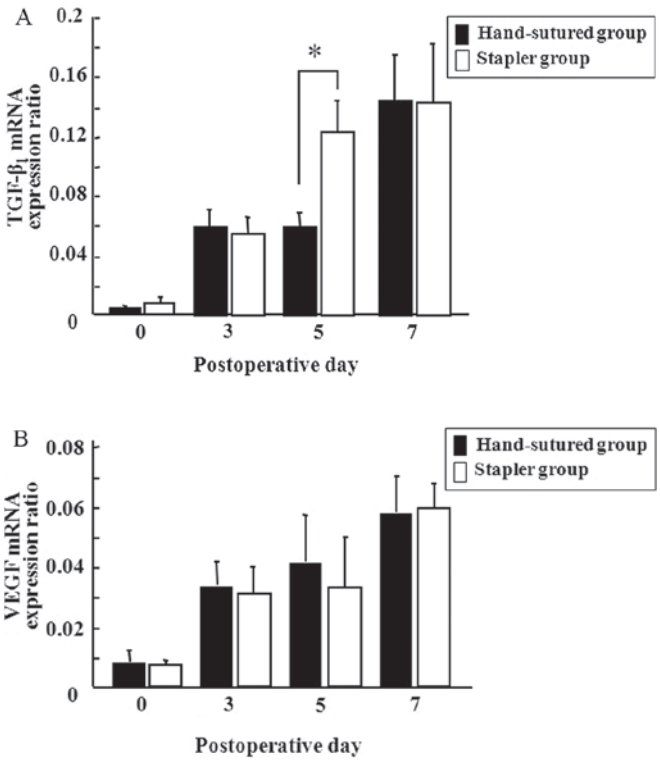

Figure 2. (A) Mean relative expression ratios of TGF-β_1 mRNA vs. GAPDH mRNA in the stapler and hand-sutured groups on postoperative days 0, 3, 5 and 7. *P<0.05. (B) Mean relative expression ratios of VEGF mRNA vs. GAPDH mRNA in the stapler and hand-sutured groups on postoperative days 0, 3, 5 and 7. Error bars represent the standard deviation of the mean.

In the gastrointestinal tract, mechanical wall strength resides in the collagenous fibrous network of the submucosa. After creation of an anastomosis, reconstitution of the submucosal layer occurs through the deposition of new collagen and degradation of existing collagen (15).

After the initial inflammatory phase, a proliferative phase ensues consisting of the formation of granulation tissue, including synthesis of noncollagenous protein such as fibronectin, and loss of colonic wall collagen in the tissue adjacent

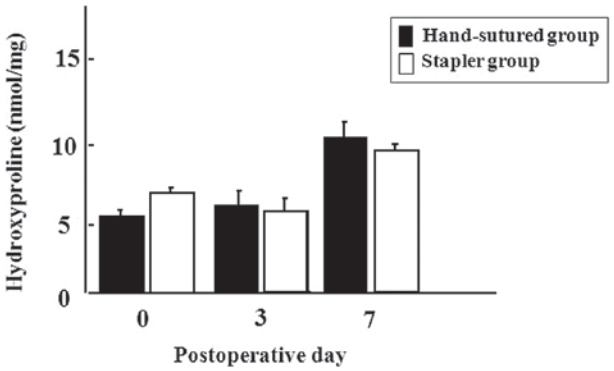

Figure 3. Hydroxyproline concentration around the anastomotic segments on postoperative days 0, 3 and 7. Error bars represent the standard deviation of the mean.

to the anastomosis (16). During the first few postoperative days, anastomotic strength is low as collagen is degraded secondary to collagenase activity at the anastomosis site. Early anastomotic strength is therefore dependent on the suture- or staple-holding capacity of the existing collagen until large amounts of new collagen can be newly synthesized by both fibroblasts and smooth muscle cells (17). Although neither type of anastomosis in the present study led to anastomotic leakage, our present results showed that mechanical staples offer better holding capacity compared to hand sutures. Ahrendt *et al* (18) found a decrease in the absolute amount of bowel wall structural collagen in intact uninjured colon following 24 h of sepsis induced by CLP. An anastomosis created under these conditions may have impaired suture holding capacity from the beginning. Therefore, we believe that the use of a mechanical stapler in conditions of peritonitis is feasible to avoid anastomotic leakage.

TGF-β_1 is an important regulator of bowel anastomosis healing. In normal bowel tissue, TGF-β_1 is mainly produced by epithelial cells (19). In injured tissues, TGF-β_1 is produced by both inflammatory cells in the lamina propria and epithelial cells (20). Migaly *et al* (21) reported that there is a crucial switch from collagen degradation to collagen deposition, with TGF-β_1 identified in wound healing as being trophic and chemotactic for fibroblasts around the POD 5. The upregulation of TGF-β_1 is temporally related to the transcription of procollagen I. The transcription of TGF-β_1 increases from the time of wounding through POD 5 and during the crucial switch from the inflammatory to the fibroplasia phase. Experimental studies have shown that in bowel anastomoses, maximum production of TGF-β_1 molecules occurs on POD 7 with subsequent diminution of TGF-β_1 levels (9). Although our results also showed this progressive increase until POD 7 in both groups, on POD 5 the stapler group showed a higher level of gene expression of TGF-β_1 than that of the hand-sutured group. We believe that in the stapler group, the crucial switch from collagen degradation to deposition may have come earlier than in the hand-sutured group because of less surgical damage resulting from less manipulation and a shorter operating time.

The formation of new vessels, or angiogenesis, is one of the key components in anastomotic healing. Vessels sprouting from capillaries, venules and arterioles help supply ischemic tissues with substrates essential for growth and repair. In an experimental analysis of angiogenesis, Seifert *et al* (22) demonstrated a significant increase in vessel growth at colonic anastomoses from PODs 3-7. VEGF is a member of the platelet-derived growth factor family of glycoproteins. It is the most potent endothelial growth factor and its role in angiogenesis is well-established (23). Upregulated by ischemia, VEGF enhances vascular permeability and vasodilatation and promotes the growth, proliferation and migration of endothelial cells (24). In the present study, gene expression of VEGF gradually increased until POD 7 and was identical in both groups. This result indicates that the degree of ischemia at postoperative anastomotic sites in both groups was homogenous, even though the sites were sutured with different material and in a different manner.

Although the concentration of hydroxyproline around the anastomotic segment was not changed on POD 3, on POD 7 an increase was observed in both groups; however, the differences were not significant. Christensen *et al* (25) reported that collagen fibrils are visible in the anastomotic healing zone after 4 days of healing and emphasized the importance of the sutures in the early healing state. Our results support their opinion and show that in our study, neither the suture material nor manner of suturing affected collagen synthesis of the healing process at the anastomosis.

In the clinical setting, many randomized studies have been performed to evaluate stapling methods in elective surgery and none revealed any difference in the incidence of anastomotic leakage (26). Particularly, a few reports have compared stapling and hand suturing in an emergency setting. Catena *et al* (27) randomized 201 patients to receive stapled or hand-sutured anastomoses and aimed to elucidate whether staplers could be used in an emergency setting and in unprepared patients. Except for significantly shorter operating times for the stapled anastomoses, no other differences were found. In our animal study, in addition to shorter operating times, we also showed that the anastomosis made by stapler is safer than that made by hand suturing in the early postoperative period in peritonitis, a type of emergency setting. In contrast to elective surgery, anastomoses in emergency surgery are usually performed in critically ill patients under difficult situations. Specifically in peritonitis, the intestine used for anastomosis is damaged by edema, congestion and intraperitoneal abscess. Even though we could not find any differences in the incidence of anastomotic leakage, we would choose the method offering higher holding capacity in the early postoperative days, the most dangerous period.

The only apparent disadvantage of stapled anastomosis is cost. Further improvements in mechanical stapling devices are necessary that can compensate for this disadvantage. Moreover, further investigation of the wound healing process, particularly the activity of PGFs, is necessary to extend the indications for stapled anastomosis in an emergency setting.

In conclusion, we found that the anastomosis made by stapler is safer and more effective than that made by hand suturing in an animal model of bacterial peritonitis as it creates a stronger anastomosis in the early postoperative period and requires less operating time. Mechanical anastomosis is feasible in intestinal surgery in the presence of diffuse peritonitis since it offers substantial holding capacity and a shorter procedure without adversely affecting the healing process.

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