

Effectiveness of gastroduodenostomy created with the use of shape memory compression anastomosis clips: observations in a porcine model

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ABSTRACT: This article evaluates the effectiveness of gastroduodenostomies created with the use of shape memory compression anastomosis clips (CAC) and compares the surgical outcomes with those of hand-sewn anastomoses. We performed Billroth's operation I in eight pigs: shape memory compression clips were used in six animals and hand-sewn anastomoses were created in two animals. Postoperative complications such as leakage or obstructed passage of digesta were not observed in any of the patients. Pathomorphological examinations and histopathological analyses confirmed that all anastomoses were tight and that gastroduodenal continuity was fully restored. In comparison with hand-sewn anastomoses, compression clips shortened the time of the surgical procedure and proved to be a safe, effective and low-cost technique for performing Billroth's operation I in animals. The experience and knowledge acquired during the experiment will be used to maximise the effectiveness of gastroduodenostomy in canine and feline patients.

Keywords: Billroth I; NiTi alloy; pig; CAC; histopathology

The concept of gastrointestinal compression anastomosis was developed nearly 200 years ago when Felix Nicholas Denans restored intestinal continuity in dogs with the use of zinc and silver rings. The 20th century witnessed a rapid development of stapling and mechanical suturing (Li et al. 2016), and interest in compression anastomosis was revived in the 1980s with the introduction of the Valtrac biofragmentable anastomosis ring (BAR) (Kaidar-Person et al. 2008). Early in this century, metal alloys with shape memory were used in the production of compression anastomosis clips. Compression anastomosis clips (CAC) are made of nickel-titanium (NiTi) alloys. Reversible martensitic transformations that take place in NiTi alloys under various temperatures enable the clip to be easily opened at low temperature (martensitic B19' phase) and to move to a closed position under exposure to body heat (B2 parent phase), thus delivering the shape memory effect (Holak and Lekston 2016).

Compression anastomosis combines two processes: necrosis resulting from the compressive force exerted by the clip on tissues, as well as healing in the direct proximity of the necrosis site (Nudelman et al. 2000; Tucker and al. 2008; Wu et al. 2014). Clips are excreted from the body with faeces. Pylorectomy and gastroduodenostomy (Billroth I) pose a challenge in veterinary practice. In dogs, the most common indications for pylorectomy are cancer and hypertrophy of the muscular layer of gastric mucosa, which prevents digesta from leaving the stomach (Frgelecova et al. 2014). Gastroduodenal leakage is the main cause of serious complications in pylorectomy and gastroduodenostomy. Manual suture is the preferred and most common anastomotic technique in gastroduodenostomy. Unlike the Valtrac BAR, the use of NiTi compression clips with shape memory for creating anastomosis in Billroth I has never been documented in human or veterinary medicine (Dietz et al. 1999). The ab-

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sence of publications discussing pylorotomy and gastroduodenostomy with the use of shape memory clips and lack of precise histopathological analysis makes such a study warranted. The aim of this study was to perform macroscopic evaluations and histopathological analyses of tissues sampled after gastroduodenostomies with shape memory compression anastomosis clips and to compare the findings with hand-sewn anastomoses.

MATERIAL AND METHODS

The experiment was performed in the Department of Surgery and Radiology of the Faculty of Veterinary Medicine of the University of Warmia and Mazury in Olsztyn in cooperation with the Institute of Materials Science of the University of Silesia in Katowice. The experiment was approved by the Local Ethics Committee for Animal Experimentation in Olsztyn pursuant to resolution No. 12/2011.

The experiment was performed on eight Polish large white pigs of both sexes; average body weight was 25 kg. The animals were divided into two groups. Group I comprised six pigs subjected to gastroduodenostomy with the use of compression clips, and group II (control) consisted of two pigs where the same procedure was performed by hand suturing.

Shape memory compression clips were made of $\text{Ni}_{50.8}\text{Ti}_{49.2}$ and $\text{Ti}_{50}\text{Ni}_{48.7}\text{Co}_{1.3}$ alloys in the form of double-coil rings measuring 25 mm × 7 mm (Figure 1). The applied clips had one-way shape memory. The clips were cooled in liquid nitrogen for 15 seconds, and were then mechanically opened to an angle of approximately 30°. When surgically inserted, clips moved to a closed position under



Figure 1. Compression anastomosis clip with one-way shape memory at room temperature

exposure to body heat. The compressive strength of clips was determined at 7–10 N under laboratory conditions.

The animals from both groups were fasted for 12 hours before surgery, and they were anaesthetised in accordance with the standard protocol for the species. A sterile surgical field was created in line with standard requirements, and abdominal integuments were incised along the midline before the level of the umbilicus. In group I animals, the pylorus was resected by closing the lumina of the duodenum and the stomach with two layers of 3-0 absorbable sutures. The antimesenteric border of the intestine was joined with the visceral surface of the pylorus with two stay sutures, approximately 20 mm from the suture closing the gastric lumen. In the next stage of the procedure, two parallel 6-mm incisions were made across all layers in the antimesenteric border of the duodenum and the pyloric part of the stomach. A cooled clip was inserted in the open position and was locked under exposure to body heat, joining the walls of the duodenum and the stomach (Figure 2). When the clip moved to a closed position, an incision was made in the joined walls of the duodenum and the stomach to restore patency of the gastrointestinal tract. The

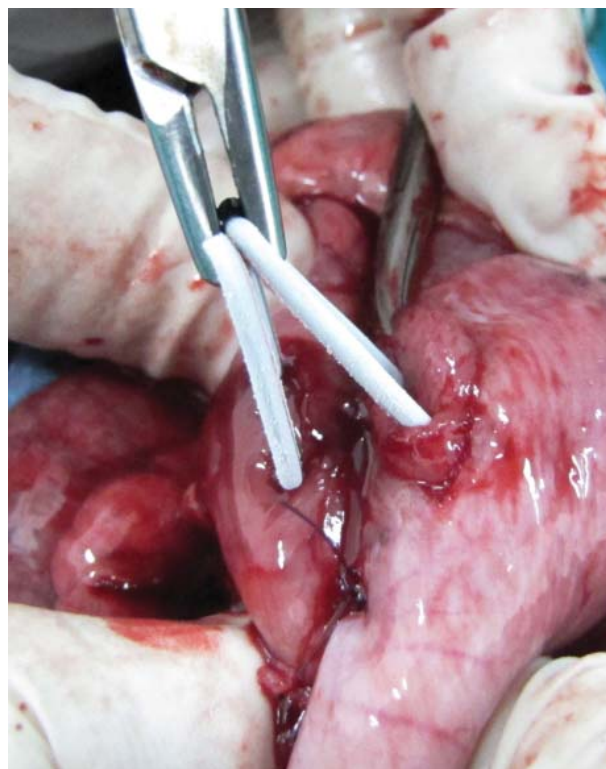


Figure 2. Insertion of a cooled and opened clip into incisions made in the gastric wall and the duodenal wall

6-mm incisions through which the clip had been inserted were closed with several single-layer interrupted 3-0 monofilament sutures. Abdominal integuments were closed with three layers of 0 absorbable monofilament sutures.

Group II animals were subjected to standard Billroth's operation I where the duodenum and the stomach were joined end-to-end with a single layer of 3-0 absorbable monofilament sutures using the Gambee technique. Abdominal integuments were closed with three layers of 0 absorbable monofilament sutures, as in group I.

The administration of feed and water began 12 hours after surgery. Antibiotics and analgesics were administered for five days. Radiological examinations of the gastrointestinal tract were performed with the use of contrast media one day and five days after surgery in all animals. Group I and group II animals were euthanised 14 days after the procedure. Gastroduodenostomies were evaluated macroscopically, and tissue samples were collected for histological analysis. Samples were fixed in 10% formalin, neutralised and buffered to pH 7.4, passed through a series of graded alcohols, purified in xylene and embedded in paraffin blocks. Microtome sections of 5- μ m thickness were stained with haematoxylin and eosin (H&E) according to the McManus method and Masson's trichrome protocol (Sigma-Aldrich, USA). For immunolabelling, the 5- μ m-thick microtome sections were mounted onto silane-coated slides. Immunohistochemical reactions were identical for all specimens. The same reagents, time, temperature and moisture conditions were used for all tissue sections. Proliferative index (PI) was determined as the number of PCNA (proliferating cell nuclear antigen)-positive cells per 100 cells (%), in five randomly selected fields of view, at 100 \times magnification. The analyses were performed on the same day using a streptavidin-biotin immunohistochemical technique specific for PCNA antibodies (DAKO, Denmark, Clone PC-10, IgG2 kappa), diluted 1 : 150. All steps were carried out in 1 M phosphate-buffered saline (PBS; 120 mM/l NaCl, 11.5 mM/l NaH₂PO₄, 31.3 mM/l KH₂PO₄, pH 7.4) at 37 °C. In the negative control the primary antibody was substituted with PBS, while commercially available human colon cancer sections (DAKO, Denmark) constituted positive controls. The microscopic evaluation was performed under a light microscope (Nikon Eclipse 80i).

RESULTS

Post-operative complications were not observed. Water and feed intake was normal in all animals. Radiological examinations of the gastrointestinal tract, performed with the use of contrast media one day and five days after surgery, confirmed the tightness and patency of the created gastroduodenostomies.

In group I, three pigs expelled clips with faeces eight days after surgery, two pigs – nine days after the procedure and one animal – 10 days after surgery.

A macroscopic evaluation performed post-mortem revealed full union of the joined segments of the GI tract (Figure 3). The mucosa was smooth and covered with small amounts of viscous mucous, without visible changes. The anastomotic diameter was determined to be 19 mm on average. In one animal, adhesion was observed between the omentum and the site of anastomosis. In group II, the created anastomoses were tight in both animals, anastomotic diameters were determined to be 14 mm and 16 mm, and adhesion between the omentum and the site of anastomosis was also observed in one pig.

A microscopic analysis revealed normal morphology of the pyloric mucosa near the site of anastomosis. The ratio of the length of gastric pits to mucus-secreting glands was determined to be 1 : 3. Gastric pits and glands were lined with a single layer of tall mucus-secreting cells. Relatively numerous intraepithelial lymphocytes were noted



Figure 3. Post-mortem view of a gastroduodenostomy created with the use of a compression clip. The site of anastomosis is marked with an arrow

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in the apical part at the outlet of gastric pits. The mucosa was covered by mucous membrane containing numerous desquamated epithelial cells. The presence of eosinophils and minor infiltration with mononuclear cells, mostly lymphocytes, was observed in the lamina propria. Thin-walled blood vessels were somewhat dilated. Single foci of mononuclear cell infiltration and oedema were noted in the submucosa and the muscular layer of the gastric wall. The proliferative index of the epithelium and lamina propria was low, approximately 30%. Masson's trichrome stain revealed numerous collagen fibres in the pyloric mucosa and a thick layer of circular and longitudinal muscles near the site of anastomosis.

Swelling of the duodenal mucosa was observed at the place of contact with the gastric mucosa. Diffuse infiltration with mononuclear cells was noted in the lamina propria of villi and at the level of intestinal crypts (Figure 4). Sparsely distributed plasmacytes and eosinophils were observed. Intestinal villi and crypts were lined with a single layer of enterocytes separated by numerous goblet cells. Large quantities of mucous with numerous desquamated epithelial cells were noted on the surface of intestinal villi. A thick layer of duodenal glands (Brunner's glands) with normal morphology was visible in the submucosa. Staining for proliferating cell nuclear antigen (PCNA) revealed a very high proliferative index of enterocytes in intestinal crypts (around 90%) which decreased towards the apex of villi (to

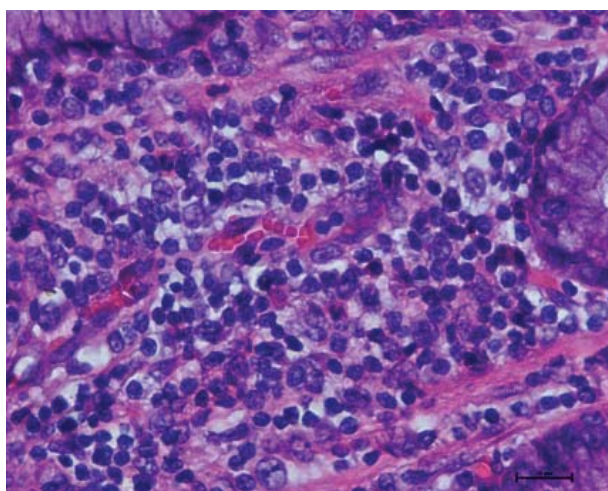


Figure 4. Infiltration with mononuclear cells (lymphocytes, plasmacytes) and sparse eosinophils in the lamina propria at the site of anastomosis. Minor dilation of vessels with swelling is visible. H&E stain

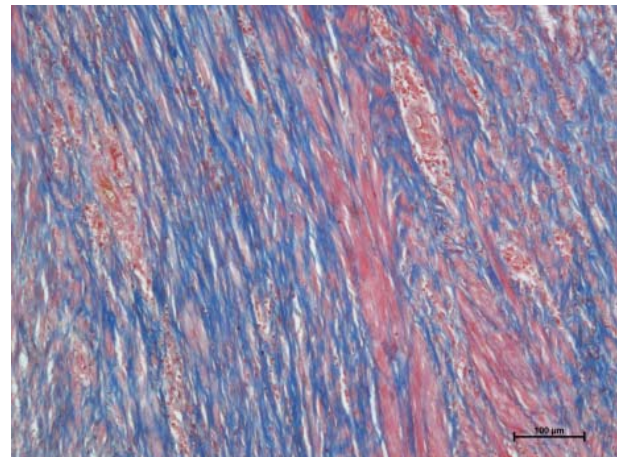


Figure 5. Dense parallel collagen fibers at the site of anastomosis with small fragments of smooth muscle tissue. Numerous fine arteries with perivascular infiltration. Masson's trichrome stain

around 50%). The mitotic activity index (MAI was determined as the number of mitotic figures per 100 cells (%), in five randomly selected fields of view, at $\times 100$ magnification) of lamina propria cells was estimated to be 20%.

Connective tissue (Figure 5) with small clusters of smooth muscle tissue and an absence of submucosa were observed at the site of anastomosis. Connective tissue was characterised by higher maturity on the side of the stomach, and it was composed of regular collagen fibrils and fibrocytes. A higher number of stimulated fibroblasts and somewhat irregular collagen fibres were noted on the side of the duodenum. Relatively numerous arteries with small diameters and minor perivascular infil-



Figure 6. Weak positive reaction for PCNA in the cell nuclei of connective tissue at the site of anastomosis

tration were observed at the site of anastomosis. Average tissue thickness at the anastomotic site was 1779.23 μm (min. 1419.63 μm , max. 2442.01 μm). Connective tissue was characterised by a low level of proliferative activity, around 15% (Figure 6).

The mean time of anastomosis, excluding surgical preparatory procedures, was 15 minutes in group I and 25 minutes in group II.

DISCUSSION

This experiment was performed on a porcine model, but dogs are the target animals for the described surgical procedure. There are two general indications for Billroth's operation I in canine patients: cancer of the pyloric segment of the stomach (Swann and Holt 2002) and hypertrophy of the muscular layers of the pylorus. In Billroth I, the most common complication which follows from the anastomosis of two sections of the GI tract with different diameters is leakage, which leads to contamination of the abdominal cavity. We suggest that shape memory compression clips open new opportunities for surgical procedures of this type.

Compression clips with one-way shape memory were used in the described experiment. A cooled clip has to be mechanically opened, but it moves into a closed position under exposure to body heat. Optimally sized clips are required for gastroduodenostomy, which is more demanding than intestinal anastomosis. The ring was 25 mm in length, and it effectively joined the gastric wall with the duodenal wall. The programmed compressive force of 7–10 N was sufficient to create a gastroduodenostomy with constant and evenly distributed pressure. Optimal compressive force should be applied to cause necrosis at the anastomotic site, facilitate the removal of the implant and create a tight connection (Nudelman et al. 2002; Holak et al. 2015). The inner diameter of the compression clip was 23 mm, and the diameter of the anastomotic site was determined to be 19 mm on average in group I animals (difference of around 1 mm between individuals) during the post-mortem evaluation. The diameter at the anastomotic site was equivalent to 83% of the clip's inner diameter. The similarities in the anastomotic diameter of group I animals indicates that the width of the created connection can be programmed depending on the size of the compression clip (Kusnierz and Lampe 2015). In

group II animals with hand-sewn anastomoses, attempts were made to create anastomoses with a width corresponding to the inner diameter of the clip. During a post-mortem analysis of group II pigs, the width of anastomoses was determined at 12 mm and 16 mm, which confirms that hand-sewn sutures can alter the anastomotic diameter, even if placed by the most highly skilled surgeon. The use of compression clips thus eliminates the risk of imprecise suturing, which is one of the major drawbacks of hand-sewn anastomoses, and the risk that the created connection will have a constricted lumen (Kusnierz et al. 2014).

In contrast to hand-sewn anastomosis, the procedure involving compression clips required only 6-mm incisions in the stomach and duodenum. Hand-sewn anastomoses require much larger incisions in both organs, which increases the risk of intraoperative and postoperative contamination of the abdominal cavity.

Compression clips were expelled with faeces eight to ten days after surgery, which is approximately one day longer in comparison with intestinal anastomoses (Holak et al. 2014). A radiological examination performed with the use of contrast media one day and five days after the procedure confirmed the tightness and patency of anastomoses and the unrestricted flow of digesta in both groups.

Macroscopic and microscopic evaluations of the created gastroduodenostomies revealed the formation of connective tissue adhesions. The described histopathological changes in the duodenal mucosa at the anastomotic site, in the submucosa and the muscular layer of the gastric wall could be attributed to the dissolution of sutures closing the intestinal wall above the compression clip. The above observation is confirmed by extensive infiltration with mononuclear cells around surgical sutures in microscopic images as well as the fact that the gastric contents normally enter the duodenum without causing changes in the duodenal mucosa. Despite the above, macroscopic and microscopic evaluations of the anastomotic site should be performed at a later date to confirm this hypothesis.

The mean time of the surgical procedure was approximately 10 minutes shorter in group I, which is an additional advantage because it reduces exposure to general anaesthesia and minimises the relevant risks for the patient. The procedure of inserting compression clips is very easy, does not require additional tools and was completed with-

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out any difficulty in this experiment. Compression anastomosis clips can also be used in laparoscopic procedures, which further extend their applicability (Kim et al. 2012).

We propose that the use of shape memory compression clips in gastrointestinal anastomosis offers a viable alternative to hand-sewn anastomosis and mechanical suturing in veterinary practice. Tight connections, a minimal number of sutures and a programmable anastomotic diameter largely eliminate the major drawbacks and imperfections of hand-sewn anastomoses. Compression clips are also significantly cheaper than other compressive anastomotic techniques (Valtrac-BAR) and mechanical staplers. The results of this study and our experience with shape memory compression clips in Billroth I indicate that this method can be safely used in clinical practice.

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