

Serosal abrasion of bowel ends does not enhance anastomotic healing



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ARTICLE INFO

Article history: Received 28 April 2014 Received in revised form 14 July 2014 Accepted 27 August 2014 Available online 2 September 2014

Keywords: Anastomosis Leakage Abrasion Serosa NSAID Diclofenac

ABSTRACT

Background: Anastomotic leakage rates remain unacceptably high, warranting reconsideration of current anastomotic technique. Anastomotic healing may improve by abrading the serosal surface of bowel ends that are invertedly anastomosed, based on the concept that serosal damage evokes inflammatory adherent processes. It is studied if local abrasion leads to stronger anastomoses and reduces leakage.

Methods: Ninety-eight Wistar rats were allocated to six groups. Either a regular anastomosis (RA) or abraded anastomosis (AA) was constructed in the proximal colon. Animals were sacrificed at day 3 (groups RA3 and AA3, $n = 2 \times 17$) or day 5 (groups RA5 and AA5, $n = 2 \times 17$). Groups RA-Dic and AA-Dic ($n = 2 \times 15$) received diclofenac from day 0 until sacrifice on day 3 to impair anastomotic healing. Outcomes were leakage, bursting pressure, breaking strength, adhesions, and histological appearance.

Results: Both in abraded (AA3 and AA5) and control (RA3 and RA5) groups without diclofenac, 1 of 17 anastomoses leaked (6%). Leak rate was 9 of 15 (60%) in group AA-Dic and 8 of 15 (53%) in RA-Dic (P = 1.0). The bursting pressure in group RA3 (127 ± 44 mm Hg) was higher (P = 0.006) compared with group AA3 (82 ± 34 mm Hg), breaking strength was comparable (P = 0.331). Mechanical strength was similar between groups RA5 and AA5. Abrasion did not increase mechanical strength in the diclofenac groups. Adhesion formation was not different between groups. Histology showed dense interserosal scar formation in abraded groups, compared with loose connective tissue in control anastomoses. *Conclusions*: Abrasion of serosal edges of large bowel ends invertedly anastomosed does not improve anastomotic strength, neither does it reduce leakage in anastomoses compromised by diclofenac.

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1. Introduction

Despite increased knowledge of anastomotic healing, leakage rates have not declined in the past decades and remain between 3 and 14% [1–3]. Leakage is a significant cause of increased morbidity and mortality after visceral surgery [4]. Attempts to reduce leakage by mechanical stapling, external sealants (e.g., fibrin glue), biological stimulants (e.g., growth factors), internal conduits, or various suture techniques have failed [3,5,6].

The current standard for constructing an end-to-end or side-to-side handsewn or stapled anastomosis is an inverting

http://dx.doi.org/10.1016/j.jss.2014.08.047

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anastomosis [7]. This means that the largest contact area of the two bowel parts is formed by the opposing serosal surfaces. However, the physiological function of the thin epithelial layer of mesothelial cells covering the serosa is to provide a lubricant surface and not to adhere. Injury of the relatively large sero-serosal contact area might provide a way to optimize anastomotic healing. When serosa is damaged, fibrous attachments may form between viscera or the abdominal wall because of the inflammatory process [8]. Serosal abrasion is the most common method to induce adhesion formation in experimental adhesion research [9]. Also for other mesothelial tissue layers, like the parietal and visceral pleura, chemical injury and promotion of the inflammatory process is used to achieve proper adherence (e.g., treatment of relapsing pneumothorax). Several methods to achieve serosal abrasion and adhesion have been described. It is mostly done by sterile gauze rubbing, but dental brushes are also used [10]. A study in dogs showed that complete removal of the mesothelium before making an inverted anastomosis accelerated and improved the healing process without an increased risk of stenosis [11]. Based on pathophysiological principles of inflammation and wound healing, it is hypothesized that isolated injury to the serosal edges of connecting bowel parts may increase anastomotic strength and reduce leak rates by stimulating fibrous adhesions between bowel ends. In the first experiment of the present study, the effect of serosal abrasion on anastomotic strength as the primary outcome was assessed. In the second experiment, it was studied if abrasion can reduce leakage of anastomoses compromised by diclofenac administration. A rat anastomosis model was used because of extensive experience with this model in our laboratory and the consistent findings of leak rates and strength over the years [12,13]. Previous research showed that the administration of diclofenac provides a reliable model to study leakage of ileal and proximal colon anastomoses [13-15]. Diclofenac causes 60%-100% leakage in the ileum and proximal colon when given from day 0 until sacrifice on day 3 [13,15].

2. Material and methods

2.1. Ethics

This experiment was conducted according to the Dutch "Experiments on Animals Act" and European Federation of Laboratory Animal Science Associations guidelines and was approved by the Institutional Animal Ethics Committee of the Central Animal Laboratory of the Radboud University Nijmegen (AEC-number 2012-290). Humane end points were defined to avoid unnecessary suffering of animals during the study.

2.2. Animals

Adult male Wistar rats (Harlan, Horst, The Netherlands) were accustomed to laboratory conditions for 1 wk and weighed 307 grams (standard deviation \pm 19) at the start of the experiment. The rats were housed two per cage at 22°C–23°C with a 12 h day cycle and had free access to standard rodent chow (Ssniff R/M-H; Bio Services BV, Uden, Netherlands) and acidified tap water throughout the experiment. The cages were enriched with a shelter and nesting material and were randomly placed on the shelves.

2.3. Groups

Ninety-eight male Wistar rats were randomly allocated to one of the six groups. Either a regular anastomosis (RA) or an abraded anastomosis (AA) was constructed in the proximal colon.

In experiment 1, animals were sacrificed at day 3 (group RA3, n = 17 and group AA3, n = 17) or at day 5 (group RA5, n = 17 and group AA5, n = 17). On day 3, anastomotic strength is at its lowest and thus most important to improve [16,17]. Day 5 was chosen as an additional sacrifice day to study if the effect of abrasion needs additional time for wound healing. Postponing sacrifice to day 7 or longer would not be useful when assessing bursting strength because anastomotic strength exceeds that of normal intestine after this period [12,13]. Three animals per group were used for histologic analysis and fourteen animals for mechanical strength testing.

In experiment 2, two groups of rats were given diclofenac (3 mg/kg/d by oral gavage; Cayman Chemical Company, Ann Arbor, MI) from day 0 until sacrifice on day 3 to induce leakage (group RA-Dic, n = 15 and group AA-Dic, n = 15) [13,15]. All animals in experiment 2 were sacrificed at day 3 because diclofenac-induced leakage occurs mostly before day 3 and postponing sacrifice would increase animal discomfort [13,15]. Twelve rats per group were used for anastomotic strength measurements and three for histologic analysis.

2.4. Intervention and surgical technique

The rats were anesthetized by inhalation of 3% isoflurane (Abbott, Hoofddorp, The Netherlands) mixed with pressurized air and oxygen. They were shaved, disinfected, and operated under sterile conditions using an operation microscope. By a 3 cm midline laparotomy, the cecum was visualized and carefully placed outside the abdomen in wet gauzes. Two centimeters distal from the cecum, the place for anastomosis was determined. In the abrasion groups, the complete circumference of the colon was abraded over a length of 2 cm with 10 soft strokes of a dental brush (Oral-B 1 2 3 Indicator Medium, Kruidvat, Nijmegen, The Netherlands) to create a precise and superficial damage of the serosal surface (Fig. 1) [10]. The middle 10 mm segment was resected, leaving 5 mm of abraded colon on both sides. In the control animals group, a 10 mm segment was removed at the same location in thee colon without abrasion. The end-to-end anastomoses were all constructed under a microscope (Wild M650; Heerbrugg, Switzerland, at \times 10 magnification) by a trained researcher (S.T.K.Y.) using a single layer of eight interrupted, inverting sutures (Ethilon 8-0; Ethicon, Norderstedt, Germany). A monofilament synthetic suture was chosen because it causes little inflammatory reaction, it is available in 8-0 size, it has produced consistent results in previous experiments, and rats are sacrificed before absorption is expected to play a role in the healing process [13,18]. The abdominal wall was closed with a running suture (Vicryl

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