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ORIGINAL ARTICLE

Anastomotic stability and wound healing of colorectal anastomoses sealed and sutured with a collagen fleece in a rat peritonitis model

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Summary *Background/Objective:* Anastomotic insufficiency is associated with increased morbidity and mortality. A collagen fleece that supports anastomosis is effective for preventing anastomosis insufficiency. The objective of this study was to compare between the stability of sutured anastomoses and that of anastomoses sealed with a thrombin/fibrinogen-coated collagen fleece in a rat peritonitis model.
Methods: In 72 male Wistar rats, peritonitis was induced with a specially prepared human fecal solution. Surgery at the rectosigmoid junction was performed 24–36 hours later. The different anastomotic techniques used were circular sutured anastomoses, semicircular sutured anastomosis and closure of the anterior wall with collagen patch, and complete closure with a collagen fleece. Bursting pressure, histology of anastomosis, mRNA expression of collagen

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types I and III, matrix metalloproteinase-13, and vascular endothelial growth factor (VEGF) were investigated after 24 hours, 72 hours, and 120 hours.
Results: All animals developed peritonitis of comparable severity. There were no differences in bursting pressures between the three suture techniques after 24 hours, 72 hours, or 120 hours. Anastomoses sealed with a collagen fleece appeared to be slightly less stable only at 24 hours, whereas they appeared to be more stable than semisutured or fully sutured anastomoses at 72 hours and 120 hours. Sealing with a collagen fleece was associated with an increase in granulation tissue, higher mRNA levels for collagen types I and III, and higher VEGF compared to sutured anastomoses.
Conclusion: The use of a thrombin/fibrinogen-coated collagen fleece showed similar efficacy to conventional sutures in colorectal anastomoses in the presence of peritonitis inflammation, and may provide additional benefits due to an increase in mature granulation tissue.

1. Introduction

Anastomotic leakage remains one of the most feared postoperative complications in colorectal surgery. It can result in anastomotic strictures, an increased risk of permanent stoma, recurrence of cancer, and mortality. In order to reduce the rate of anastomotic leakage, several studies have evaluated innovative sealing procedures, mainly using liquid fibrin glue. The overall efficacy data, however, are unconvincing.[1–3]

A collagen fleece is a tissue sealant patch composed of equine collagen, which is coated with human fibrinogen and human thrombin.[4–6] Upon administration to the wound surface, thrombin and fibrinogen form a clot, activate endogenous hemostasis, and seal the patch tightly to the wound surface.[7] Hemostasis is achieved usually between 3 minutes and 5 minutes. Efficacy in hemostasis and tissue sealing has been demonstrated in cardiovascular and lung surgery as well as in kidney and liver resection.[4,6,8–13]

Intestinal anastomoses are generally closed by sutures, but these may cause local ischemia at the anastomotic line and subsequent impairment of wound healing.[14] Suture-free bowel anastomosis using a thrombin/fibrinogen-coated collagen fleece would possibly circumvent this complication. In fact, colorectal anastomosis using a collagen fleece covered with fibrin glue components has been shown to be technically feasible in rodents[5,15,16] and pigs.[17,18] In both models, sealed anastomoses were equally efficient as the sutured ones.

Although these studies used healthy animals, peritonitis is a frequent sequel of colorectal anastomoses and is known to impair reparative collagen synthesis and wound healing.[19,20] Peritoneal soilage for 24 hours caused by cecal ligation and puncture was shown to affect anastomotic healing and wound strength in the rat colon for up to 7 days.[21]

Anastomotic bursting pressure is a well-established and frequently applied parameter used to determine the strength and sufficiency of experimental bowel anastomosis. The anastomotic character can further be determined by the presence of important tissue healing factors such as vascular endothelial growth factor (VEGF), a key mediator of angiogenesis in normal tissues[22]; collagen type I for forming mature scar tissue[23]; collagen type III; collagen of granulation tissue[23]; and matrix metalloproteinase (MMP)-13, a principal enzyme for cleaving fibrillar collagen from the endothelial matrix.[24]

The aim of this study was to investigate the effect of a thrombin/fibrinogen-coated collagen fleece on the stability and wound healing processes of colorectal anastomoses in rats with induced peritonitis by comparing three groups receiving three different closure techniques (circular sutures, semicircular sutures of the posterior wall and sealing of the anterior wall with a collagen fleece, and a complete sealing).

2. Materials and methods

2.1. Animals

Wistar WU rats (Charles River Laboratories, Sulzfeld, Germany), 12 weeks old, with body weights of 280—417 g were used. The animals were single housed in macrolon cages, were fed a standardized pellet diet, and received tap water *ad libitum*. The investigation was approved by the Schleswig—Holstein Ministry of Agriculture, Environment, and Rural Areas under license number 35/2007.

2.2. Preparation of a human fecal suspension for peritonitis induction

A rat model with standardized severity of peritonitis was established by injecting a fixed dose of a human fecal suspension into the abdominal cavity. The fecal suspension was prepared according to the procedure described by Lorenz et al[25] and Bauhofer et al,[26] with minor modifications. Fresh human stools were collected from three healthy adult individuals, and standardized fecal inoculums of each of these samples were pooled. This was mixed with thioglycolate containing 10% barium sulfate in a 1:1 ratio. The suspension was resuspended homogenously in a 1:10 ratio in phosphate-buffered saline containing 10% glycerin and 0.19 mg catalase (from bovine liver) per 100 mL suspension. The mixture was homogenized in a blender for 10 minutes to a macroscopically homogenous suspension and filtered through a sterile gauze. Aliquots of 2 mL were prepared and frozen at −80°C. Quantitative cultural analyses of representative aliquots were performed prior to freezing and after repeated thawing, to demonstrate the