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ORIGINAL ARTICLE

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Does a fibrin-collagen patch support early anastomotic healing in the colon? An experimental study

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Abstract Background Efficacy of biomaterials over intestinal anastomosis to enhance anastomotic integrity, is still controversial in clinical practice. The aim of this study was to evaluate the effect of absorbable fibrin-collagen patch (FCP) during early colonic anastomotic healing in rats. **Methods** Prepuberal Wistar albino rats were randomly divided into 6 groups of 6 rats each. Colon was transected and then anastomosed with sutures (Group A), sutures+FCP (TachoComb; Nycomed, Austria) (Group AT) or only FCP (Group T). Rats were sacrificed either 3 or 7 days after the anastomosis. Anastomoses were evaluated for perianastomotic adhesion formation, bursting pressures and histological features. **Results** Perianastomotic adhesion formation was prominent in Groups AT and T. Bursting pressures were in higher group AT than in Group A on postoperative day 3 and lower on day 7 ($p<0.05$). Histological examinations revealed an increase in inflammatory cells in Group T on day 3 and decreased wound healing in Group AT when compared to Group A on day 7 ($p<0.05$). **Conclusions** In the early period of anastomotic healing, FCP supports anastomotic integrity. However, it also causes an inflammatory reaction which may increase the time necessary for healing process. Thus, the use of this biomaterial should be preferred in only selective clinical cases with a careful follow-up.

Key words Biomaterials • Colonic anastomosis • Wound healing • Experimental study

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Introduction

Anastomotic leakage is a major complication that causes significant morbidity and mortality, especially in the early period of colonic anastomosis. In spite of opposing ideas, anastomotic complications after colon injuries are compared to complications after small bowel injuries [1–3]. None of the different surgical techniques has been found to be superior to another [2]. However, routine use of fibrin glue or omental patch support has been recommended by some surgeons to improve colonic anastomotic security [1].

Mechanical strength of wounds and scars are maintained with tissue collagen. It comprises the most important element of wound healing due to this property. TachoComb is a ready-to-use, hemostatic and sealant agent with a composition of collagen, fibrinogen, thrombin and aprotinin which is used for tissue support and hemostasis after surgical procedures. The aim of this experimental study was to evaluate the efficacy of this fibrin-collagen patch (FCP) during the early healing process of colonic anastomosis in rats.

Materials and methods

This study was approved by the local ethics committee. Thirty-six prepubertal Wistar albino rats weighing 129–185 g were used in this study. All animals were fed with standard rat pellet and

kept at room temperature on a 12-hour light and dark cycle. They were only allowed to drink water after operation.

General anesthesia was achieved with intraperitoneal ketamine hydrochloride (10 mg/100 g body weight; Ketalar, Parke Davis, Eczacibaşi, Turkey) and xylazine hydrochloride (4 mg/kg body weight; Rompun, Bayer, Turkey). For intraoperative fluid losses, 2 ml normal saline was injected subcutaneously in each rat. All animals were sacrificed with anesthetic overdose and exsanguination at the end of the study.

Surgical procedures

Rats were randomly assigned to 6 groups each composed of 6 animals:

- *Group A3*. Anastomosis with sutures assessed at day 3
- *Group A7*. Anastomosis with sutures assessed at day 7
- *Group AT3*. Anastomosis with sutures and collagen patch (TachoComb), assessed at day 3
- *Group AT7*. Anastomosis with sutures and collagen patch (TachoComb), assessed at day 7
- *Group T3*. Anastomosis with collagen patch (TachoComb), assessed at day 3
- *Group T7*. Anastomosis with collagen patch (TachoComb), assessed at day 7

After induction of anesthesia, a midline laparotomy was done and the ascending colon of the rat was found. The colon, approximately 2 cm distal to the cecum, was transected and then anastomosed. The anastomosis was done a with one-layer separated extramucosal 5/0 silk sutures in groups A3, A7, AT3 and AT7. An absorbable FCP composed of collagen derived from horse tendon, human fibrinogen, human thrombin and bovine aprotinin (TachoComb; Nycomed, Austria) was used to cover the anastomosis in groups A3, A7, AT3 and AT7. In groups T3 and T7, the anastomosis was done after approximation of the mesenteric and anti-mesenteric sides of colon with only FCP (without suture).

At the end of either postoperative day 3 or 7, the rats were anesthesized again and the anastomotic segments were isolated during relaparotomy. Integrity of the anastomosis and existence of perianastomotic abscess or peritonitis were noted, and perianastomotic adhesion formation was scored. The scoring was done as: 0, no adhesions; 1, minimal between the anastomosis and omentum; 2, moderate with involvement of omentum or a segment of intestine; and 3, severe adhesions between the neighboring intestinal segments and the anastomosis.

Clinical and histopathological analyses

Bursting pressures were measured in vivo while ligating the distal end and fixing a catheter introduced to the lumen proximal to the anastomosis. The catheter was connected to an infusion pump (Lifecare XL, Abbott Laboratories, USA) and a monitor (KMA 375, Petaş, Turkey) to measure intraluminal pressures. Through this catheter, normal saline dyed with merbisol (Mersol; Şifa Kimya, Turkey) was infused at a rate of 2 ml/min. The bursting pressure was defined as the pressure at which the leakage of the dyed saline was first noted.

After the in vivo measurement of bursting pressure, the anastomotic segment, from 1 cm each proximally and distally, was removed and fixed in formaldehyde solution. The specimens were stained with hematoxylin and eosin and the anastomoses were examined in terms of inflammatory cell infiltration (ICI), fibroblastic activity, neovascularity and collagen deposition. They were graded as: 0, none; 1, scarce; 2, minimal; 3, moderate; and 4, massive.

Statistical analysis

The results were evaluated with one-way analysis of variance (ANOVA) and post-ANOVA, least significant difference (LSD) and Tukey's tests. SPSS version 10 (SPSS, USA) was used for statistical analysis.

Results

Adhesion was noted in all groups, but in the rats in which FCP was used it was more prominent (date not shown). This difference was statistically significant between the A7 and T7 groups ($p<0.05$). No signs of perianastomotic abscess and peritonitis were noted.

On the third postoperative day, the bursting pressures were significantly higher when FCP was used over the anastomosis with sutures than when the anastomoses were made with only sutures (Table 1). On postoperative day 7, the bursting pressures were significantly higher for anastomoses made with sutures only than for those with sutures and FCP (group AT7) or with FCP only (group T7).

The anastomotic healing process was assessed histologically in terms of ICI, fibroblastic activity, neovascularity and collagen deposition. When the AT3 group was compared to A3 group according to ICI, a significant increase was noted ($p=0.004$). Fibroblastic activity was

Table 1 Anastomotic bursting pressures (mmHg), by treatment group and days after surgery, in 36 rats. Values are mean (SEM)

Surgical procedure	Bursting pressure, mmHg	
	Day 3	Day 7
Anastomosis with sutures (Groups A)	41.8 (5.9)	179.8 (20.3)
Anastomosis with sutures and FCP (Groups AT)	87.3 (11.0)*	134.8 (9.6)‡
Anastomosis with only FCP (Groups T)	71.8 (7.1)	127.0 (11.4)§

FCP, fibrin-collagen patch
*$p=0.016$; ‡$p=0.013$: §$p=0.004$ vs. anastomoses made with only sutures on the corresponding day of analysis. One-way ANOVA statistical test

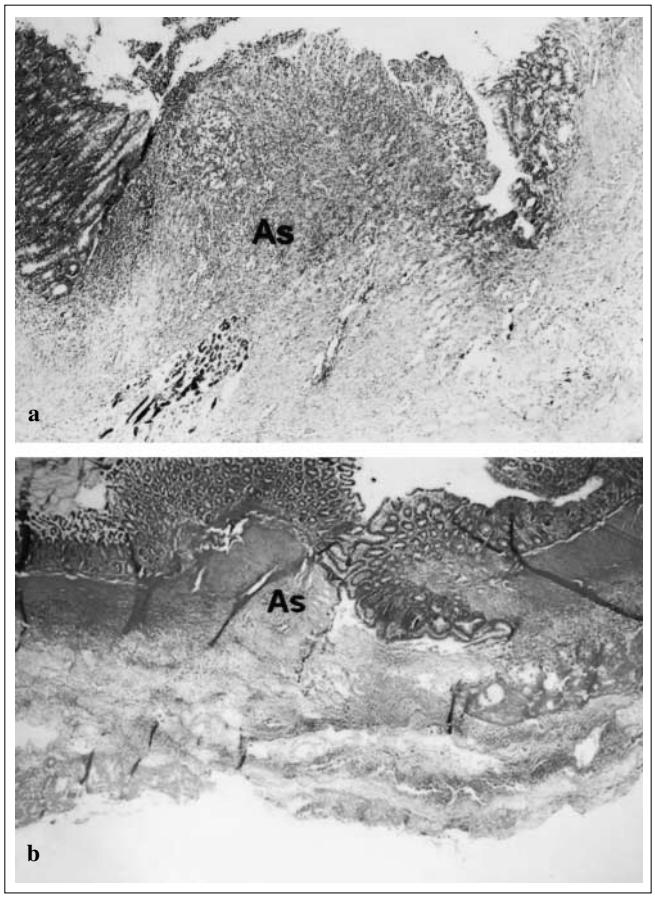

Fig. 1 Histological cross-sections of anastomotic segments in A7 (**a**) and AT7 (**b**) groups. Note the increased collagen deposition in A7. *As*, anastomotic segment. H&E, x40 magnification

increased in groups at day 7 compared to day 3 ($p<0.05$). However, the difference between the fibroblastic activities of A7 and AT7 groups was significant in favor of an increase in A7 group ($p=0.035$). Collagen deposition was not noted in the groups on postoperative day 3 but its synthesis was significant on postoperative day 7 in all groups ($p<0.05$). The increase of collagen deposition was also significant in A7 group compared to AT7 ($p=0.004$) (Fig. 1). Neovascularity of the anastomotic segment was significantly increased in A7 group compared to AT7 ($p=0.024$), and in T7 compared to AT7 ($p=0.024$).

Discussion

Anastomotic dehiscence and leakage is a feared complication after gastrointestinal anastomosis. In a large collective review, general incidence of leakage after gastrointestinal anastomosis was found to be 1.8% with a mortality rate of 24% [4]. On the other hand, overall incidence of leakage for colonic anastomosis is between 5.5% and 9% [1, 5]. Factors predictive of this complication include chronic obstructive lung disease, peritonitis, bowel obstruction, malnutrition, corticosteroids and perioperative blood transfusions in the adult population [2].

Primary anastomosis is the contemporary preferred method of repair for colonic injuries [1, 6–8]. Even under conditions of 12 or 24 hours of fecal contamination, this method is recommended for adults [9–11]. In spite of these data and other conflicting reports, experimental studies and some clinical observations have led clinicians to be precautious about colonic anastomoses [2, 12, 13]. This has increased enthusiasm for the use of different anastomotic techniques or additive materials to cover the anastomosis, such as omentum and different types of fibrin sealants [1, 14, 15]. From this perspective, the aim of this study was to evaluate the efficacy of an absorbable FCP (TachoComb) for improvement of anastomotic integrity in rat colon.

TachoComb has been used in different surgical procedures in adults and children [16, 17]. Its composition is collagen of horse tendon, human fibrinogen, human thrombin and bovine aprotinin. Fibrinogen and thrombin, upon contact with physiologic fluids like blood and lymphatic fluids, react to form fibrin monomers that stick and seal the wound surface. Collagen, in this biomaterial, achieves additive strength and aprotinin avoids early fibrinolysis. Collagen is an important component of wound healing which predicts its strength and integrity. Its amount increases in early days of healing. Good results have been reported after its use in different clinical situations but its efficacy in colonic anastomosis is yet unknown [16, 17].

Adhesion formation was observed after the use of FCP, especially in the T7 group in our study. It has also been experienced after the use of fibrin glues and sealants in other experimental studies [14, 15]. This may cause a serious problem in dealing with anastomoses under clinical conditions which can be accepted as a major drawback of this biomaterial.

FCP was found to improve anastomotic integrity on postoperative day 3, but the bursting pressures, when this patch was used, were less than those after simple suture anastomosis on postoperative day 7. There was also no significant difference between the sutured and sutureless anastomoses covered with FCP. Day 7 is the time when tissue collagen production is finalized and maturation of the healing process begins. The decreased bursting pressure during this period were unexpected as the main idea was to give additive strength with exogenous collagen. Van der Ham et al. [15] reported a reduced bursting pressure of rat colonic anastomosis 4 days after the operation when fibrin sealant was used. However, the pressures were observed to return to normal levels on postoperative day 7 [15]. The explanation for this unexpected finding was sought in the histological examination of the specimens.

Histologically, ICI, fibroblastic activity, neovascularity and collagen deposition were assessed to evaluate the anastomotic healing. ICI was prominent in groups where FCP

was used, especially on postoperative day 3. The same observation was also noted when fibrin sealants were used for improvement of colonic anastomosis [15]. The fibroblastic activity, collagen deposition and neovascularity were increased during normal healing when compared to anastomoses with the FCP at postoperative day 7. This discrepancy was projected to the decreased bursting pressure in the study groups. Thus, this patch impaired healing, of the anastomosis in spite of its support on day 3. Inflammatory phase is the first stage of wound healing, which is prominent with ICI. Increased ICI is an important finding, as these cells have enzymes called matrix metalloproteinases (MMPs) that breakdown matrix components such as collagen, which is an essential structure for anastomotic strength [3, 9, 13]. Collagen degradation starts during the initial steps of healing and it is the suture or the biomaterial that keeps the anastomosis intact during this period. MMP activity was maximal at day 3 in an experimental study on rats [9]. After this process, the production of collagen by fibroblasts takes place, which in turn restores the original strength of the healing wound [9, 13]. Foreign materials can also cause ICI followed by anastomotic leakage [14]. Peritonitis has been shown to impair reparative collagen synthesis in rats, which is a major cause of ICI itself [18]. Thus, the inflammatory cells and the MMP activity between postoperative days 3 and 7, which degraded the existing tissue collagen as well as the exogenous collagen around the anastomosis, might be responsible for the results in this study. The increased early breakdown might have affected the final amount of collagen at the end of 7 days. The significantly reduced collagen deposition when the fibrin collagen patch was used supports this explanation. However, the present study has a few limitations: silk sutures, known to incite inflammatory reaction, were used for financial reasons and collagen deposition was used to measure collagen content instead of more complex evaluations such as MMP activity, collagen ratio and degree of cross linking.

In conclusion, in the early period of anastomotic healing, FCP supports anastomotic integrity. However, it also causes an inflammatory reaction which may increase the time necessary for the healing process. This may represent a major disadvantage for this biomaterial. As a consequence, its usage should be cautiously evaluated in clinical conditions and, if preferred in selective clinical cases, careful follow-up is mandatory.

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