ORIGINAL RESEARCH

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A comparison of the application of fibrin glue and adhesive film for repair of anastomotic leaks in the rat

Abstract

Background: Anastomotic leaks constitute one of the most serious intraoperative complications and although many studies have been devoted to finding a solution for this problem, none of them has yet been able offer a decisive, successful method. In this study, the ability of fibrin glue and adhesive film to repair anastomotic leaks in an experimental model was compared.

Materials and methods: The sample comprised four groups of seven rats: Group 1 (Control): the distal colon was transected and anastomosis was performed. Group 2 (Primary repair): incomplete anastomosis produced a leak that was closed by primary repair on day 3. Group 3 (Fibrin glue): incomplete anastomosis produced a leak that was closed by primary repair and fibrin glue applied on day 3. Group 4 (Adhesive film): incomplete anastomosis produced a leak that was closed by primary repair and adhesive film was applied on day 3. The rats were sacrificed on day 6 following anastomosis. Anastomotic blast compressions were measured and fibroblast activation, inflammation, neovascularization and levels of collagen were evaluated.

Results: The results from Group 4 showed that blast compression values were high and statistically significantly increased over control values ($p < 0.05$). Inflammation in Group 2 was significantly higher than the other groups ($p < 0.05$). No significant differences were detected in the comparison of the groups regarding the other scoring criteria ($p > 0.05$).

Conclusion: Adhesive film is more effective in reducing anastomotic leakage than fibrin glue.

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Leaks developing after colon anastomosis constitute one of the most important causes of surgical morbidity and mortality. Although many approaches have been used for the repair of anastomotic leaks, no optimal treatment has yet been identified.

Some studies have investigated the effects of fibrin glues on colon anastomoses and concluded that an effective anastomotic repair can be achieved through the application of fibrin glue to the sutured area. Fibrin glues are natural hemostatic agents which contain thrombin + fibrinogen + factor XIII + aprotinin + calcium. They are used on organs that are difficult to suture, such as the liver and spleen, to stop hemostasis and secretion in areas that are difficult to reach, in nerve and vein anastomoses, parenchymatous bleeding, hemophilia and other coagulation defects, as well as many other surgical operations, and have been found to shorten the period of surgery [1,2,3,4].

Adhesive surgical film (Tissue Patch®; Tissuemed Ltd. UK) is a new surgical material in which the carboxylic acid groups provide the initial adherence via electrostatic interactions with amine and nucleophilic functionalities on tissue surfaces. Amide bonds then form rapidly between the surgical film and the tissue surface. Because the film is flexible, it adheres to the tissue leaving no gaps and completely seals the wound. It is used in the treatment of pulmonary air leaks in thoracic surgery, in dura repairs in neurosurgery and in the prevention of blood and fluid leaks in general surgery [5].

This study examines the efficacy of fibrin glue and adhesive film to repair anastomotic leaks. The stability of the anastomosis was evaluated by anastomotic blast pressure and histopathology.

Materials and Methods

This study was carried out with the consent of the Board of Ethics at Necmettin Erbakan University, Meram Medical School Hospital, and Experimental Animal Research Laboratory.

Wistar Albino female rats weighing 200-250 g (28 animals) were divided into four groups of seven. They were kept at 25°C and fed with standard rat food and normal tap water every twelve hours.

Group 1 (n = 7): Control group (Transection + anastomosis group):

The distal colon was transected at the full layer and complete anastomosis was performed with a 6/0 vicryl suture.

Group 2 (n = 7): Primary repair group:

The distal colon was transected at the full layer and sutured with a 6/0 vicryl suture. Incomplete anastomosis of the colon produced a 5 mm opening on the anti-mesenteric side. On day 3 after the procedure primary repair was carried out.

Group 3 (n = 7): Fibrin glue group:

The distal colon was transected at the full layer and was sutured with a 6/0 vicryl suture. Incomplete anastomosis of the colon produced a 5 mm opening on the anti-mesenteric side. On day 3 after the procedure, the leak was repaired with 6/0 vicryl sutures and fibrin glue (Tisseel Fibrin Sealant®, Baxter Healthcare Corp. USA) was applied to the repaired area with a 5 mm run over to both the distal and the proximal surfaces.

Group 4 (n = 7): Adhesive film group:

The distal colon was transected at the full layer and sutured with 6/0 vicryl. Incomplete anastomosis of the colon produced a 5 mm opening on the anti-mesenteric side. On day 3 following the procedure, the leak was repaired with 6/0 vicryl sutures and adhesive film (Tissue Patch®) was applied to the repaired area with a 5 mm run over to both the distal and the proximal surfaces.

Leak Model

The leak model in this study was constructed in line with previous similar studies (6). After a full transection of the distal colon, a repair was made with 6/0 vicryl suture. Incomplete anastomosis of the colon produced an opening of 5 mm on the anti-mesenteric side of the colon.

Each animal was sutured with 7-8 individual sutures at 2 mm intervals. When the leak model was developed, the continuous suture technique was not used because the aim was to form a leak on the anti-mesenteric side.

The rats in all groups were anesthetized with ketamine hydrochloride and Xylazine hydrochloride on day 6 following anastomosis.

Surgical Method

The food supply was withdrawn 12 hours before the surgical procedure, which was performed under sterile conditions by the same surgeon. The animals received an intraperitoneal injection containing a combination of ketamine hydrochloride (Ketasol 10%) 1 ml/kg (for anesthesia), Xylazine hydrochloride (Rompun 2%) 1 ml/kg (for sedation and analgesia). The front abdominal walls were then shaved and cleansed with po-

FIGURE 1. Intestinal segment repaired with fibrin glue.

FIGURE 2. Intestinal segment repaired with adhesive film.

vidone iodine prior to the opening of the abdomen with a 3 cm mid-line incision beginning under the xiphoid. When the distal colon was located, a transection incision was made perpendicular to the long axis without upsetting the blood build up in the intestinal segment. Following the transection, the control group (Group 1) underwent complete anastomosis on a single plane with a 6/0 vicryl suture. The leak groups (Groups 2, 3, 4) underwent incomplete anastomosis with a 6/0 vicryl suture, leaving a 5 mm opening on the anti-mesenteric side following the transection. The fascia and skin were closed up with a continuous 3/0 prolene suture. The animals were fed standard rat food and tap water during the post-operative period.

The fibrin glue was mixed in two separate injectors through a special mechanism and applied over the sutured area. Following the primary repair of the leak, the adhesive film was applied to the repaired area and compressed with the finger for 30 seconds.

Post-mortem colon blast pressures were measured and samples were taken for histopathological analysis of the perianastomotic tissue (Fig. 1, 2).

Although we did do any specific tests on the physical condition of the animals or other tests (eg. C reactive protein, white cell count etc) we did observe the condition of the rats during the study. The post-surgical condition of the rats in the

control group was much better than that of the animals in the leak groups, which deteriorated slowly as the study progressed. Peritoneal contamination and inflammation levels were postulated to be equal since similar inflammatory cell response was detected in rats with formed leaks.

Colon Blast Pressure Measurement

First, relaparotomy was performed on the previous incision line. The anastomotic line was located and the adhesions to the surrounding tissues were removed. After checking that the anastomotic line was secure, a 4 cm intestinal segment containing the anastomosis was resected by cutting the proximal and distal colon 2 cm on either side of the anastomosis. The distal end of the removed segment was bound with 3/0 silk and the proximal end was attached to a manual sphingomanometer after the placement of the cannula into the lumen. The intraluminal pressure was increased by immersing the colon segments in water. The blast pressure value of the initial exit of air from the leak in the repaired anastomosis was recorded and measured in mmHg.

Histopathological Analysis

The colon segments were fixed in 10% formaldehyde and analyzed by one pathologist in a blind test. The histopathological analysis of the anastomotic area was conducted according to the Erlich-Hunt model after staining in hematoxylen eosine [7]. The evaluation criteria for this model included the number of inflammatory cells, the number of fibroblasts, angiogenesis (neovascularization) and the amount of collagen (Table 1).

Statistical Analysis

Blast pressure values in the groups were compared with the Fisher Exact test and the mean values were compared with the

Table 1. Scoring for the Erlich–Hunt model of colonic anastomosis

Scoring	Inflammatory cell/Fibroblast Neovascularization/ Amount of collagen
1	In small quantities but sporadic
2	In small quantities and in all areas
3	In large quantities but sporadic
4	In large quantities and in all areas

Table 2. Anastomotic blast pressure values of the groups

Experimental Animals	Group 1: Control (mm/Hg)	Group 2*: Primary Repair (mm/Hg)	Group 3**: Fibrin Glue (mm/Hg)	Group 4**: Adhesive Film (mm/Hg)
1	80	20	50	90
2	100	20	40	300 ^b
3	70	0 ^a	90	150
4	60	30	80	120
5	100	20	100	120
6	90	10	90	300 ^b
7	80	0 ^a	120	130
Mean	82.86	14.29	81.43	172.86
Std. deviation	14.96	11.34	27.95	88.64

^a Animals whose anastomotic lines burst spontaneously

^b Animals whose anastomotic lines remained intact at 300 mm/Hg).

* Significant ($p < 0.05$) with Fisher Exact test and Mann Whitney U test. Group 2 had lower blast pressure values than groups 1, 3 and 4.

** Significant ($p < 0.05$) with Fisher Exact test and Mann Whitney U test. Group 4 had higher blast pressure values than Group 3.

Mann Whitney U test. For this histopathology, the Bonfemori correction was applied in the statistical analysis. The SPSS program was used for analyses.

Results

Blast pressure

Blast pressure data are summarized in Table 2. Group 2 had lower blast pressure values than the other groups ($p < 0.05$). There was no significant difference between Group 1 and Group 3 ($p < 0.05$). Group 4 had higher blast pressure values than the control group (Group 1) ($p < 0.05$) and higher blast pressure values than Group 3 ($p < 0.05$). The anastomotic repairs in two rats in Group 2 burst open before blast pressure was applied. In Group 4, it was observed that the anastomotic repairs in two rats remained intact although 300 mmHg pressure was applied. In summary, repairs using either fibrin glue or adhesive film resulted in a stronger anastomotic repair. Anastomoses repaired with adhesive film were stronger than those repaired with fibrin glue. Although there were no leaks in the control group, the adhesive film group proved to have a more stable anastomosis.

Histopathology

Results are summarized in Table 3. Group 2 had more inflammatory cells than Group 3 and Group 4 ($p < 0.05$). Surpris-

ingly, Group 1 (control group) showed no significant difference in inflammatory cells in comparison with all other groups. There were no statistically significant differences in fibroblast activation, neovascularization or amount of collagen among the groups ($p < 0.05$).

Discussion

Anastomotic leaks in colorectal surgery are associated with significant morbidity and mortality and may result in poor functional and oncological outcomes. Diagnostic difficulties may delay the identification and appropriate management of leaks [8].

There are many reports of clinical and experimental studies devoted to the research on anastomotic wound healing but although some systemic and local factors have been named, there is still no definitive treatment method that will reduce the rate of leaks and accelerate wound healing in high-risk cases. Among the methods currently being used in the diagnosis of anastomotic leaks, sonography, X-ray, fluoroscopy and CT are effective, but despite a variety of both clinical and radiological diagnostic methods, there is still no standardized classification [9]. Komen et al. state some risk factors regarding the causes of anastomotic leaks, including diverticular disease, rectal resection, emergency interventions, smoking, high body mass index, steroid abuse, radiotherapy or chemotherapy, a high ASA score, a history of cardiac or vascular surgery, anas-

TABLE 3. Histopathological evaluation of all groups according to the Erlich – Hunt model and score percentages.

Evaluation	Score	Group 1	Group 2*	Group 3	Group 4
Inflammatory Cells*	2	71.6%	0%	42.9%	42.9%
	3	14.2%	42.9%	57.1%	57.1%
	4	14.2%	57.1%	0%	0%
Fibroblast Activation	1	42.9%	57.1%	42.9%	42.9%
	2	57.1%	42.9%	57.1%	57.1%
Neovascularization	1	0%	0%	14.2%	0%
	2	28.4%	57.1%	43.1%	57.3%
	3	71.6%	42.9%	14.2%	28.5%
	4	0%	0%	28.5%	14.2%
Amount of Collagen	1	71.6%	85.8%	71.6%	57.1%
	2	28.4%	14.2%	28.4%	42.9%

*Significant ($p < 0.05$) with Fisher Exact test and Mann Whitney U test. Group 2 had more inflammatory cells than group 1, 3 and 4.

tomosis performed manually or with a stapler and duration of the operation [10]. The question remains as to what surgical strategy in the treatment of anastomotic leaks results in the best outcome. By comparing primary repair techniques in an experimental model, we aimed to resolve the problem of how to reduce the risk of complications regarding anastomotic repairs performed in a non-sterile environment with standard information and skills and following basic surgical principles. Our strategy was to support the anastomosis externally using fibrin glue and adhesive film. Based on our clinical experience and knowledge of the literature, it is clear that primary repairs performed in an infected and edematous environment will not be successful.

Fibrin glue is a biological adhesive material made from concentrated fibrinogen, which can be used as an adhesive in wound repair. It is a water-resistant cover and can thus constitute a physical barrier around the anastomosis. Clinical reports indicate that it might prevent anastomotic leakage after colonic operations. Kanellos et al. reported that the application of fibrin glue around a sutured anastomosis reduced the rate of anastomotic leaks and strengthened the anastomosis [11,12]. In another study by Subhas et al., the authors used a combination of fibrin glue and Gentamicin [13]. The results of the histopathological analyses with blast pressure indicated that the strength of anastomosis increased as a result of the application of fibrin glue and Gentamicin [13].

The results of our study show that the application of fibrin glue and adhesive film increased anastomotic blast pressure

values significantly ($p < 0.05$), although values were very low in the primary repair group. These results are, therefore, consistent with the above-mentioned studies. Stoma application is also among the methods frequently used in anastomotic leaks of the colon. The use of fibrin glue and adhesive film in anastomotic leaks may reduce stoma indications in colorectal surgery. Opening up stoma will both prolong the period of hospitalization and increase morbidity, in addition to creating various other health problems. Following anastomosis done in an intensively inflammatory and edematous environment, the techniques used in our study appear to increase the strength of this anastomosis. The statistically significant improvement in the anastomotic blast pressure indicates that more clinical and experimental investigations need to be done. A larger number of experimental animals and repetition of these experiments with different materials will shed new light on the subject.

Another parameter used in this study was histological evaluation. There were no statistically significant differences between the groups in this regard. The anastomotic repairs were performed in a non-sterile environment, which explains the high number of inflammatory cells. Contrary to our expectations, there was no statistically significant difference between the control group and the leak groups regarding the number of inflammatory cells ($p > 0.05$). A possible explanation for this is that the anastomosis in Group 2 involved primary repair only and was weaker than the others, and also in groups 3 and 4, the anastomosis was supported with fibrin glue and adhesive film.

Omentoplasty has been used as a treatment strategy for leaks observed after colon and rectal surgery but a randomized, multi-centered study carried out on this subject reported that the results were not successful [14]. Our study, however, did yield significant differences, especially for the group treated with adhesive film when compared with the control group ($p>0.05$).

Adhesive film is indicated for use in sealing and reinforcing against air leakage in thoracic surgery, especially in lung resection, and also against leakage of low pressure bleeding or oozing of blood or fluid leakage following surgical procedures on soft tissue, such as the liver, in splenic resection and splenic injury. Adhesive film is intended for use as an adjunct in thoracic and general surgery and is not intended to replace sutures or staples. Studies of patients with pulmonary air leakage, comparing the use of fibrin tissue glue and adhesive film, report that the use of adhesive film is more effective in reducing leakage and drainage than fibrin glue [5,15]. There may be other clinical applications for this product but, as yet, it is very new and there are no published reports of it being used for any other purpose – such as for prevention of intra-abdominal adhesion. In addition, there is no study in the literature examining the use of adhesive film to repair anastomotic leaks. The results of our study demonstrate that the adhesive film group (Group 4) had statistically significantly higher values of anastomotic blast pressures than the other groups ($p>0.05$), including the fibrin glue group (Group 3) ($p>0.05$). It is clear then, that the use of fibrin glue and adhesive film following the primary repair of anastomotic leaks can be used to support leak repair; however, according to the results of our study, the repairs done with adhesive film appear to be stronger. The use of adhesive film is more advantageous because of the relative ease of preparation (no special mechanism is required for mixing or application as with fibrin glue) and the rapid application.

The high rates of failure in surgical procedures on edematous tissues and the resulting conditions of morbidity and mortality constitute a significant problem. Repairs with adhesive film can be used to address this clinical setback and thereby reduce potential complications.

Conclusion

Edema and inflammation on the intestinal wall make re-repair difficult. In our study we used fibrin glue and adhesive film in addition to primary repair to support anastomosis in rats with anastomotic leaks. The results of our study demonstrate that repairs using either fibrin tissue glue or adhesive film are stronger than those with primary repair alone. It was also observed that anastomosis supported by adhesive film was more

durable than that using fibrin glue. We did not, however, observe an association between differences in anastomotic blast pressures and histopathological findings. Finally, the use of adhesive film could be an important step in achieving a durable repair in anastomotic leaks.

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