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Effects of Epidermal Growth Factor on Colonic Anastomoses: An Experimental Study

Güneş ME[1], Çakar E[2], Bayrak S[2], Deniz MM[3] and Çağlıkülekçi M[4]*

[1]Department of General Surgery, Istanbul Esenyurt University, Turkey

[2]Department of General Surgery, Istanbul Research and Training Hospital, Turkey

[3]Department of General Surgery, Bayrampasa State Hospital, Turkey

[4]Department of General Surgery, Yeniyuzyıl University, Turkey

Abstract

Purpose: This study investigated the effect of Epidermal Growth Factor (EGF) on reducing anastomotic leak and wound-related complications in rats following colonic anastomosis.

Methods: Wistar-albino male rats of 14-16 weeks and weighing 200-250 grams. First, the rats were equally randomized into a control and an experimental group. Then, in each of these two groups three subgroups were formed, each consisting of five rats that were sacrificed on the postoperative 3rd, 7th and 21st days. Using an insulin injector, a 10 microgram/kg dose of EGF was administered to the rats in the experimental group, first submucosally on the anastomosis region during the operation, then intraperitoneal at the postoperative 12th, 24th and 48th hours.

Results: Biomechanical, histopathological and clinical examinations were conducted to evaluate the wound healing in the subjects. The bursting pressure and tensile strength of anastomosis were measured in both the control and EGF-administered groups and a comparative analysis was performed. The results of both parameters were found to be significantly higher in the EGF-administered groups than in the control group.

On the postoperative 3rd day, an anastomosis leak was observed in one rat from the control group and the bursting pressure was measured as 0 mmHg. In the EGF-administered groups; the histopathological examination of the wound on the postoperative 3rd day showed higher values of serum fibrin, fibrocytes and fibroblast. On the postoperative 7th day; the granulation tissue was further developed and the granulation distance increased and on the 21st day; the strength of the granulation tissue was higher.

Conclusion: We conclude that administering EGF to subjects following colonic anastomosis has positive effects on wound healing.

Keywords: Colon; Anastomosis; Epidermal growth factor; Leakage; Bursting pressure; Tensile strength

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Correspondence:
Mehmet Emin Güneş, Department of General Surgery, Istanbul Esenyurt University, Turkey, Tel: +90 212 4449123; Fax: +90 212 6990990; E-mail: memgunes@hotmail.com

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Introduction

A wound is the disruption of anatomical and functional continuity of living tissue. Even though the basic characteristics of wound healing are similar in all tissues, there are certain differences in the gastrointestinal system. For example, the recovery of tensile strength is slower in dermal wounds than in colon wounds [1]. In the latter in addition to fibroblasts, smooth muscle cells also produce collagen [2], and the collagen synthesis in fibroblasts from colon and skin has different mechanisms [3]. In the gastrointestinal tract, there are also different factors; such as the presence of a wide range of microorganisms, the effect of serosa on suture closure [4].

Epidermal growth factor (EGF) was first reported by Stanley Cohen as a factor that promotes wound healing [5]. EGF is effective in stimulating the migration and division of epithelial cells and increasing the synthesis of proteins (such as fibronectin) that lead to cell adhesion and migration. In addition EGF increases the production of other growth factors and improves their effect on the cells [6].

Fogue-Lafitte et al. [7] reported that there are EGF receptors in the epithelial cells on the basolateral surface of intestinal villi. In vivo studies have also shown that the intravenous administration of an

Table 1: Bursting pressures and tensile strength of anastomoses in all groups.

Groups		Anastomotic Bursting Pressure (Mean)		Anastomotic Tensile Strength (Mean)	
Group 1 (Post-Op Day 3)	Control	35.00 mmHg	z: 1.68 p: 0.009	128.00 gr	z: 2.43 p: 0.015
	EGF	72.00 mmHg		140.00 gr	
Group 2 (Post-Op Day 7)	Control	145.00 mmHg	z: 2.63 p: 0.009	182.00 gr	z: 2.19 p: 0.028
	EGF	210.00 mmHg		212.00 gr	
Group 3 (Post-Op Day 21)	Control	180.00 mmHg	z: 2.62 p: 0.009	260.00 gr	z: 1.05 p: 0.095
	EGF	225.00 mmHg		280.00 gr	

To compare the bursting pressure and anastomotic tensile strength between the subgroups (3rd, 7th and 21st days) statistical measurements were performed using the Kruskal-Wallis test. The difference between all groups was found to be statistically significant (Table 2).

Table 2: A comparison of bursting pressures and tensile strength of anastomoses in all groups.

Group		Day	Mean Score	X^2	P
Control	Bursting Pressure	Day 3	3.00		
		Day 7	8.20	12.08	0.002
		Day 21	12.80		
	Anastomotic Tensile Strength	Day 3	3.00		
		Day 7	8.00	12.50	0.002
		Day 21	13.00		
EGF	Bursting Pressure	Day 3	3.00		
		Day 7	8.50	11.48	0.003
		Day 21	12.50		
	Anastomotic Tensile Strength	Day 3	3.20		
		Day 7	9.00	9.64	0. 008
		Day 21	11.80		

increased dose of exogenous EGF results in mucosal growth in the small intestine and colon [8].

The exogenous administration of EGF has been considered to promote early and stronger wound healing. Due to the higher number of pathogenic microorganisms and increased collagenous enzyme activity, the risk of an anastomotic leak in the colon is higher than in the small intestine. It is predicted that the positive effects of EGF on wound healing can reduce anastomotic complications and result in faster and more reliable healing of anastomosis.

This study investigated the effect of EGF on wound healing in the colon through histopathological, physical and clinical assessments.

Material and Method

The subjects of this study were 30 Wistar-albino male rats of 14-16 weeks and weighing 200- 250 grams. During the course of the experiment, the guidelines in the Declaration of Helsinki with regard to the care and use of laboratory animals were strictly followed. The subjects were first divided into a control and an experimental (EGF-administered) group, each consisting of 15 subjects. Then, three equal subgroups were formed within both groups according to the day of sacrifice; 3rd, 7th, and 21st days following resection and anastomosis.

After overnight fasting, general anesthesia was performed on all the rats with the subcutaneous administration of 5mg/kg Xylazine HCL and intramuscular administration of 50 mg/kg Ketamine HCL. The abdominal area of each rat was shaved and disinfected with povidon iodine and then a median laparotomy was performed. A 0.5 cm segment of colon within 5 cm of the ileocecal valve was resected and then end-to-end anastomosis with single layer inverted 5/0 Vicryl sutures was performed. The abdominal wall was closed in a single layer using 2/0 stitches using the en block technique.

Prior to the dilution of EGF; the physiological saline was kept in the freezer at -18°C and then the diluted EGF was stored at +4°C. Using an insulin injector, a 10 microgram/kg dose of EGF containing the saline solution was administered to the subjects in the experimental groups; first submucosally on the anastomosis region during the operation, then intraperitoneal at the postoperative 12th, 24th and 48th hours (EGF, Sigma Immuno Chemicals Company, USA).

The same surgeon and pathologist performed all the operations and histopathological assessments, respectively.

For the evaluation of the bursting pressure, the distal end of the anastomosis was closed using 3-0 silk sutures. A 3 mm diameter polyethylene catheter was placed in each subject at the proximal end of the anastomoses using 3-0 silk sutures and a Riester aneroid manometer was used to measure the pressure. The colon segments were placed in a transparent container filled with saline and the pressure was gradually increased. Anastomoses were observed and the air bubbles appearing on or near the anastomotic line were recorded as the bursting pressure.

To evaluate the tensile strength, a total of 5 cm segment of the colon on the anastomotic region with 2.5 cm proximal and distal margins of the wound was used. A fixed clamp was placed 2.5 cm above the anastomosis, the distal end was ligated using 2-0 silk sutures, and the area was clamped. Then, the urinary catheters were filled with physiological saline at the rate of 60 cc/min from a height of 50 cm, and the tensile strength was recorded when a rupture occurred on the anastomotic line.

For the histopathological examination, all tissues were paraffin embedded and 0.5-micron sections were stained with Hematoxylin–Eosin (H-E) dye. The sections were analyzed under light microscopy and the images were transferred to a computer. Analyses were conducted by a single pathologist who did not have any knowledge of the groups. The wound healing along the colon anastomotic line was evaluated under light microscopy on the 3rd, 7th and 21st days. The parameters of the presence of fibrin, neovascularization, inflammatory infiltration, fibroblast activity and collagen formation were evaluated using van Gieson staining. The fibroblast activity was assessed based on the presence and density of young fibroblasts within the granulation tissue. The collagen presence was determined according to the density based on (+++).

The anastomosis bursting pressure and tensile strength were evaluated using the Mann-Whitney- U test and Kruskal-Wallis test.

Results

In this study; all 30 rats were evaluated. The three subgroups of

rats in each of the control and experimental groups were weighed, sacrificed and necropsied on the 3rd, 7th and 21st days following surgery.

An anastomotic leak was observed in one subject in the control group at the end of the 3rd day. Anastomotic colon segments with leakage were separated and the bursting pressure was 0 mmHg.

In all the groups (3rd, 7th and 21st days) the anastomotic bursting pressure and tensile strength were analyzed using the Mann-Whitney-U test and the EGF-administered groups were found to have significantly higher values when compared with the control groups (Table 1).

Histopathological examination

Day 3: Fibrin, neovascularization and inflammatory infiltration along the anastomotic line were evaluated in both the control and EGF groups. The fibroblast activity in the EGF-administered group was found to be more prominent (Figure 1).

Day 7: Partially organized granulation tissue developed on the colon anastomotic line. A small amount of neovascularization and inflammatory infiltration was observed. The fibroblast activity was found to be (++) in the control group and (+++) in the EGF group (Figure 2).

Day 21: The granulation tissue on the colon anastomotic line was organized but the fiber development was not substantial. In the EGF-administered group, collagen, elastic and reticular fibers were observed as well as the granulation tissue that was gradually reduced. Proliferous fibrocytes and fibroblasts, histiocytes, new vessel formations, low-density polymorphs and high-density lymphocytes and the presence of plasmocytes were noticeable (Figure 3).

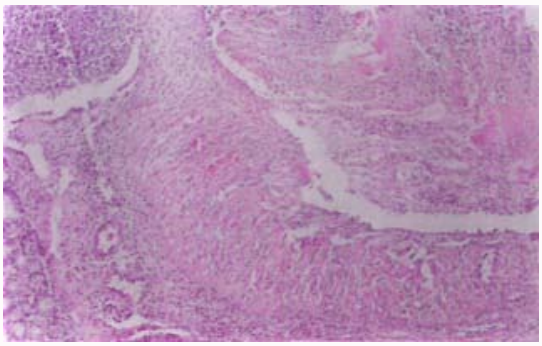

Figure 1: Fibrin, neovascularization and inflammatory infiltration along the anastomotic line.

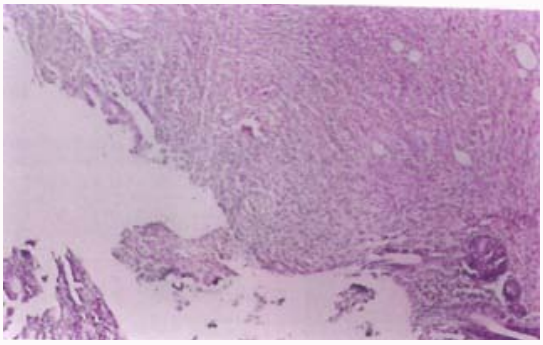

Figure 2: Partially organized granulation tissue developed on the colon anastomotic line.

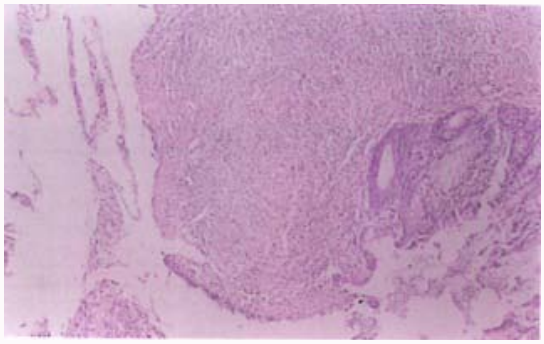

Figure 3: The granulation tissue on the colon anastomotic line was organized.

Discussion

Anastomotic leaks have significant negative effects on patient mortality and morbidity, oncological results and healthcare expenses. Despite the recent medical improvements, the incidence of anastomotic leak still ranges from 2.5 to 37%. Therefore, the development of new techniques and materials for an optimum anastomotic recovery continues to be an appealing area of surgical research [9,10].

Physiological anastomotic healing proceeds *via* an overlapping pattern of events that can generally be divided into three classic stages of wound repair: an exudative phase, a proliferative phase and a reparative phase [11]. Peptide growth factors have been reported to have a significant role in many stages of this process. In the literature, various studies have been conducted and different pharmacodynamics methods have been used to demonstrate the positive effect of peptide growth factors on the recovery of intestinal anastomosis which is a complex biological process [10,12-17].

The EGF family is probably the most commonly used factor in wound healing with EGF being one of the most researched factors. EGF is secreted by platelets, macrophages and fibroblasts and acts in a paracrine fashion on keratinocytes [18]. The mitotic effect of EGF has been reported to increase cell division and neovascularization, epithelial cell migration, experimental wound healing in the gastrointestinal system and the tensile strength of incisions [6].

Johnson et al. [19] conducted a study on rats to determine the breaking strength at different times following bowel anastomoses and concluded that sutures significantly contribute to healing. The authors reported that the strength of a new anastomosis was 2/3 of that of a non-operated gut wall and within three postoperative days, the anastomotic strength was reduced to the 15% of the immediate postoperative value. The authors concluded that this showed a rapid decrease in the suture holding capacity in the early postoperative period. The rapid increase after the 4th day and the strength of anastomosis being higher on the 7th day than the immediate postoperative value were attributed not only to the synthesis and deposition of collagen but also to the gut wall regaining the capacity to withstand breaking forces.

Kingsnorth et al. [20] investigated the effect of EGF on the recovery of gastrointestinal anastomoses. They measured the tensile strength of stomach, ileum and colon on the postoperative five day and found that the intraperitoneal administration of EGF increased the wound strength in all incision types. Ekiz et al. [6] also explored the effects of EGF on intestinal anastomosis, fascia and skin wound healing.

They experimentally created gastrojejunostomy anastomosis in rats, intraperitoneal administered EGF at 8th, 16th, 24th and 36th hours, sacrificing the subjects on the postoperative 3rd, 7th and 21st days. The authors found that the anastomotic tensile strength was significantly higher only in the EGF group on the 3rd postoperative day when compared to the tensile strength of the control group. In the present study, we subserosally injected the EGF into the perianastomotic area during the operation and performed intraperitoneal injections at postoperative 12th, 24th and 48th hours. We found the tensile strength of colon anastomosis to be significantly higher on the postoperative 3rd, 7th and 21st days when compared to the control group.

Hermann et al. [21] evaluated the anastomotic area in terms of bursting pressure and reported that the anastomotic strength against the lumen pressure was slightly noticeable on the 3rd postoperative day and exhibited no significant change on the 4th and 5th postoperative days. The bursting pressure from the 7th day onwards was found to be equal to the intestinal strength and the maximum strength was observed between the 8th and 10th days. The bursting pressure is important for anastomosis since it is a parameter that demonstrates the resistance of the intestinal wall to the increase in the intraluminal pressure. The effects of a number of chemical, physical, and nutritional elements have been investigated on the normal or inhibited wound healing process of colonic anastomosis including arginine, short chain amino acids, vitamin A, zinc, He: Ne laser, erythropoietin and hyperbaric oxygen [14]. The increase of bursting pressure values of the controls were 32% with short chain fatty acids [22], 24% with arginine [23], 37% with erythropoietin [24]. A study by Sakallioglu *et al.* [14] concerning local EGF administration indicated a 22% higher of bursting pressure than the controls in non steroid inhibited wound healing. In the present study, we found the bursting pressure in the EGF group to be significantly higher than in the control group; 100% higher on the 3rd day, 45% higher on the 7th day and 25% higher on the 21st day.

Wound healing in the gastrointestinal system depends on a thin balance between collagen synthesis and collagenolysis. This balance is even more significant between the 3rd and 5th postoperative days. The most effective layer on the colon tensile strength is submucosa. Collagen synthesis in the submucosa provides a mechanical structure for the wound [25]. Fibroblasts begin the collagen synthesis 24 hours after injury. Since there is also collagen breakdown, a significant deposition does not occur before the 3rd or 4th day. This corresponds to the first and real breaking strength of the wound [26]. In the present study, the histopathological examination showed that the serum, fibrin, fibroblast and polymorph levels in the anastomotic area were higher in the EGF group on the 3rd day and the results obtained from the 7th day indicated that the intensity of the inflammatory tissue gradually increased. On the 21st day, an increase was observed in the number of collagen, elastic and reticular fibres.

There are various factors that may have an effect on anastomotic wound healing; such as old age, accompanying diseases, medication, emergency interventions, infection, hypotension, long surgery time, inexperience of the surgeon, patient's diet, the vascularity of the anastomotic line, tensile strength and the anastomotic technique. However, only the last three are under the control of the surgeon [27]. In their study on growth factors and gastrointestinal anastomotic healing, Rijcken et al. [10] suggested that despite still being far from clinical routine in gastrointestinal surgery, growth factors can be used in selected patients who face the risk of impaired anastomotic healing.

Conclusion

We conclude that supplementing sutured anastomoses with EGF can be very beneficial in colon anastomoses cases both for the patients at risk of inadequate wound healing and for surgeons to feel more confident about the procedure particularly when anastomosis is technically difficult to perform. However, further investigation should be undertaken with a larger number of subjects and longer-term observation to extend and improve the clinical applicability of the use of the EGF factor.

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