Does locally applied epidermal growth factor stop anastomotic leak in the colon?

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AIM*: Anastomosis leakage is one of the most common complications after colorectal surgery. Studies have shown that the incidence of anastomotic leakage is between 0.5-30%. The aim of our study was to investigate the efficacy of local application of of epidermal growth factor (EGF) on colon anastomosis healing.*
MATERIAL AND METHODS: *28 Wistar rats were randomly divided into 4 groups. Sham group, control group, saline injection group, EGF injection group. Anastomosis line was determined as 3 cm distal to ilealcecal junction. The rats were reoperated on the 7th postoperative day. The colon segment was cut out 3 cm proximal and distal to the anastomotic line.The bursting pressure of each removed colon segment was measured and the segments were fixed with 10% formaldehyde for pathology examination. Anastomosis line was stained with hematoxylin eosin and histopathological evaluation was performed. Evaluation parameters were inflammatory cells, fibroblast, angiogenesis (neovascularization) and collagen amounts.*
RESULTS: *Bursting pressure was higher in the EGF group than in the control group and saline injection group. There was statistically significant difference between EGF and positive control group. (p<0,05) Histopathological examination revealed that the inflammatory cell density was higher in the positive control group than in the other groups. Fibroblast cell density, neovascularization and collagen content were higher in EGF group than the others. However, no statistically significant difference was found between the control group,saline injection group and EGF injection group.*
CONCLUSION: *As result of our study,we think that local application of EGF may have a positive effect on healing of colon anastomosis.*

KEY WORDS: Colonic Anastomosis, Egf, Experimental, Healing

Introduction

The leakages of colonic anastomosisis one of the major complications of colorectal surgery with high mortality and morbidityrates. Several factors, such as localized infection, inappropriatesurgical technique, bowel ischemia, obstruction at the distal of the anastomosis and tension in the anastomosis line have been identified as the major reasons influencing the healing of the anastomosis[1]. Despite many systemic and local factors affecting the healing of the anastomosis line, new parameters and factors are required to investigate the healing of the colonic anastomosis.For this reason,many experimental rat models have been used to identified effects of pharmacological agents on colonic healing [2-5]. But nothing has been found that has been accepted for local application to the anastomosis area. The aim of our study

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was to investigate the efficacy of local application of epidermal growth factor (EGF) on colon anastomosis healing growth factors are one of the most popular drug due to important roles in thedevelopment, repair, cellular differentiation and cell proliferation of the intestine [6-9]. It has been used in wound healing since two decades, non-experimental studies have not been reported yet.Therefore, we examined the effects of local application of EGF on experimental colon anastomoses as growth factors have implications for wound healing.

Material and Methods

This study was approved by the Local Ethical Committee of Animal Experiments of 9 Eylül University(protocol-no. 26/2019). In this study, 28 Wistar rats weighing 200-270 gr were used. All animals were kept in a 20-22 °C temperature room with a light-dark cycle and with 50-60% relative humidity in standart animal room. Rats were fed on *ad libitum* to standart chow and water. Throughout the study, daily weight monitoring, feed and water consumption per cage were monitored.it was divided into four groups containing seven rats.

Surgical Procedure

Anaesthesia was induced by administration of 45 mg/kg ketamine intraperitoneally (Alfamine %10, Ata fen) and 5 mg/kg xylazine (Xylazinbio 2%, Bioveta). Abdominal hair was shaved, skin was sterilised with 10% povidone iodine.Laparotomy was performed via a midline incision and the colon and ceacum was taken out of the abdomen. The colon was transected from 3 cm distanced to ileoceacal junction. Afterwards, anastomosis was performed using interrupted 4/0 silk sutures (Doğsan, İstanbul) in a single-layer, end-to-end technique (Fig. 1). Fascia and skin were closed using continuous 3/0 monofilament sutures (Katsan, İzmir). All anastomoses performed by the same surgeon. Subsequently, 5 ml of sodium chloride and fluid replacement was performed subcutanously. Rats were fed with standart chow and water until the 7th postoperativeday. Rats were sacrified on the 7th day. The reason for choosing this colon segment is that the density of the microbiota in this part of the colon is very high and consequently local infection is an important factor among the factors affecting the healing of the colon anastomosis.

Experiment Groups

Group 1 (Sham group): Laparotomy was performed but transection and anastomosis was not performed.
Group 2 (Positive control group): After laparotomy, colon transection was performed. Afterwards, colocolonic anastomosis was performed with 4/0 silk sutures.
Group 3 (Saline injected group): After colon transection, a total amountof 0.05 cc of saline in was injected into the intramural areas of proximal colon segment (medial and lateral points) and distal colon segment (anterior and posterior points) from 4 points with a 26 G needle in equal doses. Afterwards, colocolonic anastomosis was performed with 4/0 silk sutures.
Group 4 (EGF-injected group): After colontran section, 2 µg / kg EGF (heberprot-p 75 mg), was injected into the intramural areas of proximal colon segment (medial and lateral points) and distal colon segment (anterior and posterior points) from 4 points with a 26 G needle. İn equal doses.Afterwards, colocolonicanastomosis was performed with 4/0 silk sutures.

Assessment Methods

All rats under went laparotomy under anesthesia 7 days after the first operation. The adhesions were separated with fine and blunt dissections with out damaging the anastomosis line.
Segmental colectomy was performed by cutting the colon segment from 3 cm proximal and distal to the anastomosis line. The inside of the removed colon segment was cleaned by saline washing.

Fig. 1: End-to-end colon anastomosis.

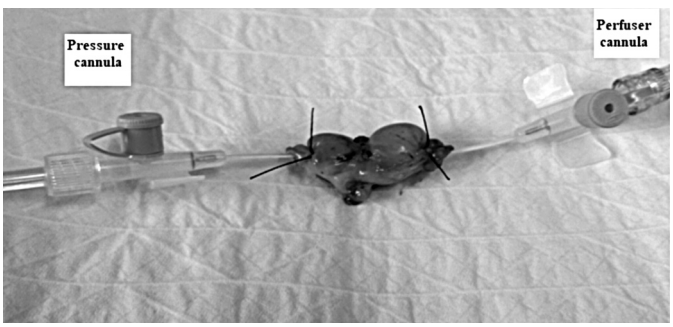

Fig. 2: Anastomosis burst pressure measurement.

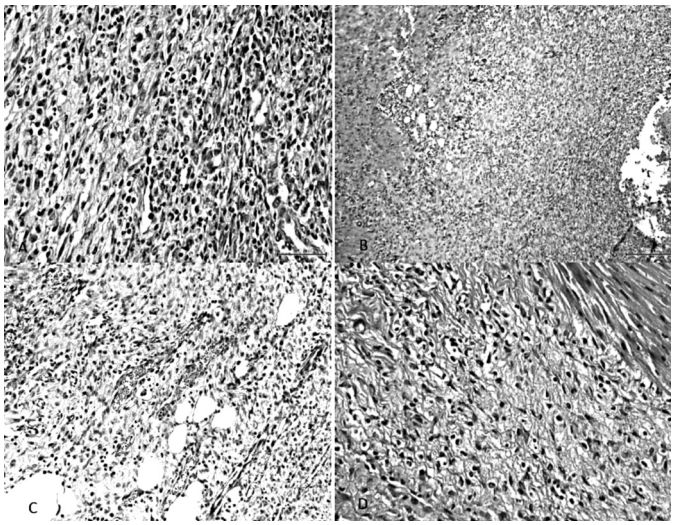

Fig. 3: Histopathological evaluation of the anastomosis line of the grups (A, C, D 400x magnification, B 200x magnification).

Anastomotic Bursting Pressure

Bursting pressures were measured on postoperative 7th days in all rats.The distal ends of all resected anastomotic colon pieces were securely tied using 3/0 silk suture.A polyethylene catheter was placed into the lumen at the proximal end for saline infusion and another catheter attached to a transducer placed at the distalend. A set-up required to view the intraluminal pressure in millimeter of water (mmH_2O) was thus achieved.
Saline was infused into the colonic lumen at arate of 2 ml/min. The first saline leakage from the anastomotic line was documented as the anastomotic bursting pressure (Fig. 2). Bursting pressure calculated as 2 diastolic pressure + 1 systolicpressure / 3 = average pressure. Groups were compared over calculated mean bursting pressure.

Histopathological Evaluation

Colon segments were fixed with 10% formaldehyde and stained with hematoxylin-eosin then evaluated by a single pathologist. Erlich- Hunt model was used for histopathological evaluation of the anastomosis line. In this model, inflammatory cell accumulation, fibroblast cell density, collagen deposid and neovascularization were evaluated. Scoring was done as follows (Fig. 3).
Score 1: Small amount, present in a scattered pattern;
Score 2: Small amount and present in all areas;
Score 3: High amount, present in a scattered pattern;
Score 4: High amount and present in all areas.

Statistical Analysis

The data obtained in the study were analyzed using SPSS (Statistical Package for Social Sciences) for Windows 25.0 program. Descriptive statistical methods (number, percentage, mean, Standard deviation) were used while evaluating the data. Since the number of data is low, non-parametric testing was preferred without normality analysis. In the analysis of the data, (ANOVA test nonparametric equivalent) Kruskal Wallis H test was used in comparison of quantitative data which were more than two categorical in the measurements. However, multiple comparison tests were performed to find different group. Relations between categorical variables were investigated by Chi square analysis. $P < 0.05$ was considered statistically significant

Table I - *Lenght of interval between the treatment procedures.*

Patient#	LALA/EE(Days)	EE/Resection (Days)	Completion of all Procedures (Days)
1	12	12	24
2	30	6	36
3	22	11	33
4	23	38	61
5	17	13	30
6	19	11	30
7	11	37	48

EE, Endovascular exclusion of the aneurysm; LALA, Laparoscopic lavage/drainage

Table II - *Multiple comparison test results of inflammatory cell accumulation, fibroblast cell density, neovascularization and collagen deposit values.*

Compared Groups	Inflammatory cell density (p)	Fibroblast density (p)	Neovascularization (p)	Collagen deposid(p)
Grup1/Grup2	0.0	.009	.007	.015
Grup1/Grup3	.005	.009	.046	.015
Grup1/Grup4	.019	.002	0.0	.001
Grup2/Grup4	1.0	1.0	1.0	1.0
Grup3/Grup2	1.0	1.0	1.0	1.0
Grup3/Grup4	1.0	1.0	1.0	1.0

Results

Only one subject from the sham group died within 30 minutes postoperatively duringthe 7-day period from the beginning of the experiment to the end.

Bursting pressures: The burst pressure medians of control and study groups were 166 (min-max:151-180mmH^2O), 141 (min-max:136-162 mmH_2O), 148 (min-max:137-156 mmH_2O), 164 (min-max: 150-169 mmH20), respectively. The results show a statistically significant difference between the positive control group and EGF injected group ($p = 0.029$), sham group and the positive control group ($p = 0.05$), sham group and the saline injected group ($p = 0.035$).

There were no statistically significant results between the group with EGF injected and the saline injected group ($p = 0.171$), between the group with EGF injected and the sham group ($p = 1.0$), between the positive control group and the saline injected group ($p = 1.0$).

Histopathological evaluation score: When the multiple comparison test results of the values of inflammatory cell accumulation,fibroblast cell density, neovascularization and collagen deposid were examined, statistically significant results were found between the sham group and positivecontrol, saline injected group and EGF injected group ($p <0.05$) (Table I).

When the other groups were evaluated among themselves, it was found that the inflammatory cell density was lower in the EGF injection group than the other groups, the fibroblast cell density, neovascularization, and collagen deposid were higher.

There was no statistically significant difference between the other groups (Table II).

Discussion

Anastomotic leakage represents a major complication in colon surgery with high impact outcomes on the patients' morbidity and mortality. Some of the factors that cause leakage in colon anastomoses demonstrated by clinical and experimental studies 10. Despite great progress in the field, the rate of anastomotic leakage after colonic resections still higher up to 37%. Therefore, further development of anastomotic healing has been of great interest in surgical research.

The healing of anastomosis is aprocess in which many type of cells go to the wound area and work cooperatively to repair the wound as a result of injury. The types and amounts of inflammatory cells in the anastomosis area, neovascularization, and collagen fiber densities provide information for the evaluation of the safety of the anastomosis line [11-12].

Today, although there are many experimental and clinical studies using locally or systemically administered substances to reduce anastomosis leakage, no systemic or local substance has been put into clinical use. A number of studies have investigated the beneficial effects of growth factors among the items used. One of these items, EGF was first isolated in 1962 by Dr. Stanley Cohen from salivary gland of mice [13]. The proposed benefits of EGF have been attributed to a variety of biological effects, including the migration, proliferation, induction of extracellular matrix synthesis and angiogenesis effects. There are many studies showing that EGF positively affects the healing of mucous wounds and causes an increase in tensile strength in intestinal wounds [14-17]. The findings suggest that EGF may be an important protein that plays a role in new collagen-making conditions such as colon anastomosis healing by increasing fibroblast proliferation [18]. In a study in which systemic administration of growth hormone was performed, the subjects were divided into two groups. Segmentary colon resection was performed in both groups, and then colonic anastomosis was performed. Until the 6th postoperative day, one group was given saline injection while the other group was injected with GH. The subjects were sacrificed on the 7th postoperative day. The anastomosis line was resected, and burst pressures were calculated and histopathological evaluation was made. As a result of the evaluation, it was determined that the burst pressure was significantly higher in the GH applied group compared to the other group. Also, in the GH applied group, inflammation decreased, but neoangiogenesis, fibroblast activity and collagen deposition increased [19]. In a study model in which EGF was applied to the ileum anastomosis and intraperitoneally, although the bursting pressures were low in the saline group,no significant difference was found with EGF. In the group in which EGF was both injected and intraperitoneal infusion was performed, it was found that the bursting pressure was statistically significantly higher [20]. In an experimental study investigating the effect of EGF on esophageal anastomosis, the bursting pressure was foundto be higher in EGF group compared to control, and it was found that fibroblast prophylaxis was more intensive after the 21st day while there was no statistically difference between groups in inflammation and neovascularization [21]. Similarly, in a study, investigating the local effects of EGF, it was found that the anastomotic bursting pressure was higher on the 7th day when compared with the anostomosis of EGF-impregnated sponge group and the gelatin sponge group [22]. In another hybrid study, while EGF was injected into the anastomosis line during the operation, then intraperitoneally administered at the postoperative 12th, 24th and 48th hours.

As a result, on the 3rd, 7th and 21st days, the study groups showed high bursting pressure compared to the control groups, while the fibroblast activity was found high on the 3rd and 7th days 23. In a study where EGF was applied to the colon anastomosis line, the bursting pressure was found to be high on the 7th day and it was found to be statistically significant. It was found that the inflammatory cell densities were low in

the EGF group and were not significant in terms of collagen and vascular proliferation [24].
In our study, the examination was made on the 7th day. The mean pressure in the EGF injected group was higher than the positive control and saline injected groups, but this difference was statistically significant only in the positive control group. This result is similar to other studies.In histopathological evaluation, inflammatory cell density appeared higher in the control group.Although fibroblast density, neovascularization,and collagen density were high, EGF injected group but results were not statistically significant. These findings seem to be compatible with other results [21-24].

Conclusion

As a result, we think that the EGF have been shown to be involved in healing processes in various cells groups including the early period of the anastomotic healing.The high bursting pressure in the anastomosis line is a positive effect of EGF. This opens the perspective to use EGF therapeutically to support anastomotic healing.In addition, although we found the beneficial effect of EGF on fibroblast, neovascularization and collagen deposid, we did not find a significant difference compared to control groups.
We believe that local EGF application will make more difference, especially in the presence of factors that delay wound healing.Maybe taking into account the results of this study, we believe that studies to be formed with control groups known to have wound healing problems will reveal the difference more clearly.

Riassunto

La discenza anastomotica è una delle complicanze più comuni dopo l'intervento chirurgico di resezione colorettale. Gli studi hanno dimostrato che l'incidenza delle deiscenze è compresa tra 0,5 e 30%. Lo scopo del nostro studio era di indagare l'efficacia dell'applicazione locale del fattore di crescita epidermico (EGF) sulla guarigione dell'anastomosi del colon.
Materiale e metodi: 28 ratti Wistar sono stati divisi casualmente in 4 gruppi. Gruppo sham, gruppo di controllo, gruppo iniezione salina, gruppo iniezione EGF. La linea di anastomosi è stata realizzata a 3 cm distalmente alla giunzione ileao-cecale. I ratti sono stati rioperati in 7a giornata postoperatoria. Il segmento del colon è stato ritagliato a 3 cm prossimale e distale alla linea anastomotica. È stata misurata la pressione di scoppio di ciascun segmento del colon rimosso e i segmenti sono stati fissati con formaldeide al 10% per l'esame istologico. La linea di anastomosi è stata colorata con ematossilina eosina ed è stata eseguita la valutazione istopatologica. I parametri di valutazione adottati sono stati cellule infiammatorie, fibroblasti, angiogenesi (neovascolarizzazione) e quantità di collagene.
Risultati: la pressione di scoppio è risultata maggiore nel gruppo EGF rispetto al gruppo di controllo e al gruppo di iniezione salina. C'era una differenza statisticamente significativa tra EGF e il gruppo di controllo positivo ($p<0,05$). L'esame istopatologico ha rivelato che la densità delle cellule infiammatorie era maggiore nel gruppo di controllo positivo rispetto agli altri gruppi. La densità cellulare dei fibroblasti, la neovascolarizzazione e il contenuto di collagene erano più elevati nel gruppo EGF rispetto agli altri. Tuttavia, nessuna differenza statisticamente significativa è stata trovata tra il gruppo di controllo, il gruppo di iniezione di soluzione salina e il gruppo di iniezione di EGF.
Conclusione: come risultato del nostro studio, riteniamo che l'applicazione locale di EGF possa avere un effetto positivo sulla guarigione dell'anastomosi del colon.

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