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RESEARCH ARTICLE

# The Effects of Perianastomotic Injections of Different Growth Factors on Experimental Colonic Anastomoses in Rats

## Çeşitli Büyüme Faktörlerinin Deneysel Kolonik Anastomoz Yapılan Ratlarda Anastomoz Etrafına Enjekte Edilmesinin Etkileri

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## ABSTRACT

**Aim:** Despite advances in medicine and technology, anastomotic healing problems and leaks are still the most important causes of postoperative morbidity and mortality in colorectal surgery. Growth factors are proven to have significant effects on wound healing. The aim of the present study was to determine the effects of different growth factors on experimental colonic anastomoses and the healing process.

**Method:** The study included 48 Wistar rats, weighting 200-250 g, which were divided to 6 randomised groups (n=8): Group I-Sham group; Group II-Partial colostomy+colonic anastomosis; Group III-Partial colostomy+colonic anastomosis+125 µg/kg epidermal growth factor; IV-Partial colostomy+colonic anastomosis+6.5 µg/kg keratinocyte growth factor; V-Partial colostomy+colonic anastomosis+16 µg/kg fibroblast growth factor; Group VI-Partial colostomy+colonic anastomosis +150 µg/kg granulocyte-colony stimulating factor. All factors were injected subserosally to the perianastomotic area. On the postoperative 7$^{th}$ day, rats were sacrificed and perianastomotic tissue examples were obtained for colonic bursting pressure, tissue hydroxyproline levels and histopathologic examination.

**Results:** Colonic bursting pressures were higher in Groups III and V compared to Groups II and VI ($p<0.01$). Tissue hydroxyproline levels also were higher in Groups III, IV and V compared to Groups II and VI ($p<0.001$). Histopathologic examination revealed that the healing parameters were higher in Groups III, IV and V, and lesion parameters were higher in Groups II and VI.

**Conclusion:** This study suggests that local application of epidermal, keratinocyte and fibroblast growth factors in the anastomotic area improves the healing process of colonic anastomoses.

**Keywords:** Experimental, healing, colorectal

## ÖZ

**Amaç:** Sağlık ve teknoloji alanındaki tüm gelişmelere rağmen; kolorektal cerrahide anastomoz iyileşme problemleri ve kaçaklar halen ameliyat sonrası morbidite ve mortalitenin en önemli sebepleri olarak görünmektedir. Büyüme faktörleri yara iyileşmesine yönelik anlamlı güçlü etkileri olması sebebiyle denemektedir. Yürütülmekte olan bu çalışmanın amacı; çeşitli büyüme faktörlerinin deneysel kolon anastomozları ve yara iyileşmesi üzerine etkilerini incelemektir.

**Yöntem:** 200-250 gr ağırlığındaki 48 Wistar türü rat, 6 randomize gruba bölünmüştür. Grup I-Sham grubu, Grup II-Parsiyel kolotomi+kolonik anastomoz, Grup III-Parsiyel kolotomi+kolonik anastomoz+125 µg/kg epidermal büyüme faktörü, Grup IV-Parsiyel kolotomi+kolonik anastomoz+6.5 µg/kg keratinosit büyüme faktörü, Grup V-Parsiyel kolotomi+kolonik anastomoz+16 µg/kg fibroblast büyüme faktörü ve Grup VI-Parsiyel kolotomi+kolonik anastomoz+150 µg/kg granülosit-koloni uyarıcı faktör. Bütün faktörler anastomoz alanına subserozal olarak enjekte edilmiştir. Ameliyat sonrası 7. günde ratlar sakrifiye edilmiş ve anastomoz etrafı doku örnekleri kolonik patlama basıncı, doku hidroksiprolin düzeyi ve histopatolojik inceleme amaçlı toplanmıştır.

**Bulgular:** Kolonik patlama basınçları Grup III ve V'te Grup II ve VI'ya nazaran yüksekti ($p<0,01$). Doku hidroksiprolin düzeyleri de Grup III, IV ve V'te Grup II ve VI'ya oranla daha yüksekti ($p<0,001$). Histopatolojik inceleme sonuçları iyileşme parametrelerinin Grup III, IV ve VI'da daha yüksek olduğunu; lezyon parametrelerinin Grup II ve VI'da daha yüksek olduğunu göstermiştir.

**Sonuç:** Bu çalışma epidermal, keratinosit ve fibroblast büyüme faktörlerinin lokal uygulanmasının kolon anastomoz iyileşme sürecini geliştirdiğini iddia etmektedir.

**Anahtar Kelimeler:** Deneysel, iyileşme, kolorektal

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## Introduction

Despite the advances in technics, technology and suturing materials, comparing with surgical procedures applied to other organs, colorectal surgery is still one of the most complicating surgical procedure worldwide. Leakage that's seen after colonic anastomoses increase the patient's morbidity and mortality.[1] It has been reported that when the anastomosis is closer to the anus, leakage rate is higher in elective colorectal surgery.[2] Various factors, including preoperative using of steroids, elongation of the operation time and contamination of the surgical area; increase the anastomotic leakage rates.[3]

Recent studies notify that sufficient blood flow is essential for healing of colonic anastomoses and adequate oxygenization improves collagene synthesis and increases the safety of the anastomoses.[4,5,6,7]

Wound healing process is composed from inflammation, collagene accumulation and collagene maturation steps. Blood vessels are the most important elements of this early stage of trauma responce.[8]

The resistance of the anastomosis against streching and other factors is dependent on collagene.[9,10] Bowel wall contains collagene type I, III and V.[11] Collagene catabolism is higher than synthesis in first postanastomotic day, but in seventh day, thus collagene synthesis passes the catabolism.[12] Wound healing is controlled by a variety of regulators.[13] Experimental studies have shown that growth factors (GF) demonstrate considerable effects on wound healing.[14,15] These factors regulate the cell functions, including growing, differantiation and metabolism.[15] Each GF acts on different step of wound healing: e.g. inflammation, matrix synthesis and storage, angiogenesis, epithelisation and contraction.[14,15]

Epidermal GF (EGF) is a mithogenic polypeptide, containing 53 aminoacides, appears in many different tissues and body fluids of mammals.[16] It has mitogenic properties on epithelial and mesothelial cells.[17] EGF; exhibits strong action on cell division and differantiation both in vivo and in vitro by binding to own glycoprotein receptor, which is localised on the cell surface of fibroblasts, cornea, lence, small and large intestine, glia and epithelial cancer cells.[18]

Fibroblast GF (FGF) is a member of a wide polypeptide family and plays role in the regulation of the cell growing and differantiation.[19] FGFs demonstrate mithogenic, chemotactic and angiogenetic effects on mesodermal cells, which are very important in tissue repair and regeneration. FGF-1, (acidic FGF) and FGF-2 (alkaline FGF) are the leading members of this family.[20,21]

Keratinocyte GF (KGF) is a member of the FGF family and is entitled as FGF-7.[22] This factor is fairly specific for keratinocytes and induces dermal fibroblasts in case of skin wounds within 24 hours.[23] KGF is a 28 kDa single chain polypeptide and is secreted by stromal cells of almost all epithelised organs. KGF effects the epithelial cells by inducing proliferation, migration and morphogenesis.[22]

Granulocyte-colony stimulating factor (G-CSF) is a 17 kDa glycoprotein. Recent studies showed that; the most of the G-CSF receptors are expressed on neutrophils and a little part on monocytes in vitro. G-CSF is used routinely in afebryl and febryl neutropenia due to chemotherapy, acute myeloid leukemia, myelodysplastic syndrome and bone marrow transplantations.[24]

Determination of the effects of perianastomotic injection of different GFs on colonic anastomoses in rats composes the goal of this study.

## Materials and Methods

**Anesthesia and Technique:** The present study was performed in Medical and Surgical Research Centre Laboratory of the Eskişehir Osmangazi University Faculty of Medicine after approval of the Local Ethics Commitee.

A total of 48 male Sprague-Dawley rats, weighting 200-250 g, were housed at constant temperature with 14/10 h periods of light and dark exposure, respectively. In acclimation period of at least five days prior to experiments, animals were allowed to access standard rat chow and water ad libitum. Rats were starwated 12 hours before experiments and randomized into six groups (n=8). After anesthesia with subcutaneous injection of 50 mg/kg thiopental sodium (Pental Sodyum, İE Ulagay, Turkey) rats were fixed in supine position, shaved and disinfected with povidone-iodine 10% (Isosol, Merkez Laboratory&Medical Tic., İstanbul, Turkey). Dry operating field covered with sterile drape and median laparotomy was performed. Except for sham group, all contents of caecum and accending colon were pushed distaly and a partial colotomy was performed on accending colon. Thereafter all colotomies were closed with single layer separated 7/0 polypropylene sutures. Sham group rats were subjected only to colonic exploration. Experimental GFs were injected with 26 G syringes in subserosal area of the anastomotic region in groups III, IV, V and VI. Rats were divided into six randomised groups (n=8):

I. Sham group (Grup S): colon exploration

II. Colon anastomosis group (control) (Grup CA): anastomosis of ascending colon

III. EGF + Colon anastomosis group (Grup EGFCA): 125 µg/kg EGF (SIGMA 2008, Germany).

IV. KGF + Colon anastomosis group (Grup KGFCA): 6,5 µg/kg KGF (SIGMA 2008, Germany).

V. FGF + Colon anastomosis group (Grup FGFCA): 16 µg/kg FGF (SIGMA 2008, Germany).

VI. G-CSF + Colon anastomosis group (Grup CSFCA): 150 µg/kg G-CSF (Roche 2008, Turkey).

After closure of the abdomen, animals were placed into separated cages and after 12 hours from operation allowed to oral feeding. On the 7th postoperative day relaparotomy was performed in the same fashion and after sampling of 2 cm proximal and distal perianastomotic area of the colon rats were sacrifised with decapitation.

**Measurement of Colonic Bursting Pressure (CBP):** The feces in sampled colonic lumen was cleaned. After placing of a catheter into proximal edge of the colon both egdes were closed with 2/0 silk in an effort to avoid air leakage. The uncovered edge of the catheter was connected to a standart sphygmomanometer and infussion pump through a fluid infussion set. Prepared colonic segment was placed into a glass container, filled with normal saline. By means of infussion pump, the intracolonic pressure was increased with air, infused with 6 mL/min velocity. The value of sphygmomanometer at the moment of detection of air bubles was recorded as CBP.

**Measurement of Tissue Hydroxyproline (THP) Level:** Following CBP measurement, the colonic segment was cutted 1 cm proximaly and distally to the anastomosis.

A 2 cm long colonic segment, containing the anastomosis at median point was acquired. This segment was cutted vertically to 2 equal segments, each segment have anastomosis at the median point. One of this segments was placed in 10% formalin solution for histopathologic examination and another was freezed immediately at-700 C for subsequent THP level measurement.

A ready-maid purchased hydroxyproline estimation kit was used for THP level measurement (HypronosticonR, Organon, HOLLAND). By inserting the obtained optic densities into below formulation, the THP level was calculated as mg/L: 2 x N tube's optic density x 50/S tube's optic density-N tube's optic density.

By estimation and calculation of used tissue weight and added fluid amounts, this THP level was converted to THP consentration. The results were expressed as µg hydroxyproline/mg tissue.

**Histopathologic Examination:** From tissue samples in formalin solution paraffin blocks were prepared, then 4.5 micron in tickness sections were dyed with Haematoxylin-Eosin (H&E) and analysed blindly by a single pathologist. Tissues were assessed in terms of alterations leading to healing of the anastomoses (healing parameters) [i.e. vascular proliferation (VP), collagene tissue proliferation (CTP), fibrous tissue proliferation (FTP), and mononuclear leukocyte infiltration (MNLI)] and in terms of alterations leading to impairment of the anastomoses (lesional parameters) [i.e. mucosal ulseration (MU), perianastomotic oedema (PAO), polimorphonuclear leukocyte infiltration (PMNLI)].

Determined results were scored from 1 to 4 as follows: 0 to 25% changes as 1, 26 to 50% changes as 2, 51 to 75% changes as 3 and 76 to 100% changes as 4.

### Statistical Analysis

CBP and THP levels were analysed using One-way ANOVA test, histopathological scores were analysed using Kruskal-Wallis test and multiple comparisons of both analyses were performed using Tukey's method.

## Results

### Colonic Bursting Pressure Levels

Comparison of CBP levels of the groups revealed statistically significant difference ($p<0.001$). CBP levels of the Group S was significantly higher than all of other groups ($p<0.001$). Comparing the anastomosis groups; CBP values of the EGFCA and FGFCA groups were significantly higher in contrast to Group CA ($p<0.01$ and $p<0.05$, respectively). There was no diffference between Groups KGFCA, CSFCA and Group CA ($p>0.05$) (Figure 1).

### Tissue Hydroxyproline Levels

There was significant difference between THP levels of the groups (F: 28.05; $p<0.001$). Accordingly, THP levels of Group S was significantly lower comparing with other groups, except Group CA ($p<0.001$). Comparing the anastomosis groups; the values of Groups EGFCA, KGFCA and FGFCA were higher than Group CA ($p<0.001$, $p<0.001$ and $p<0.01$, respectively). There was no difference between Group CSFCA and Group CA ($p>0.05$) (Figure 2).

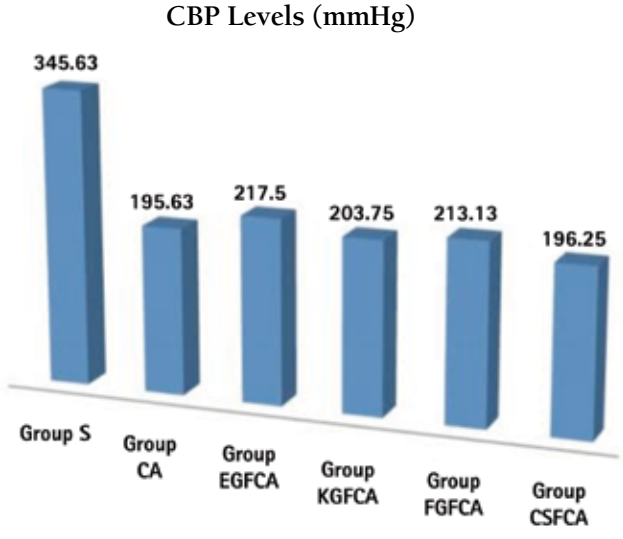



**Figure 1.** The distribution of colonic bursting pressure levels between groups (values are given as medians)
CBP: Colonic bursting pressure, CA: colon anastomosis

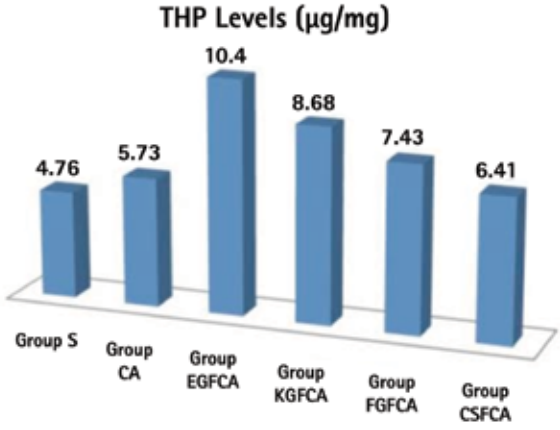



**Figure 2.** The distribution of tissue hydroxyproline levels between groups (values are given as medians)
THP: Tissue hydroxyproline, CA: colon anastomosis

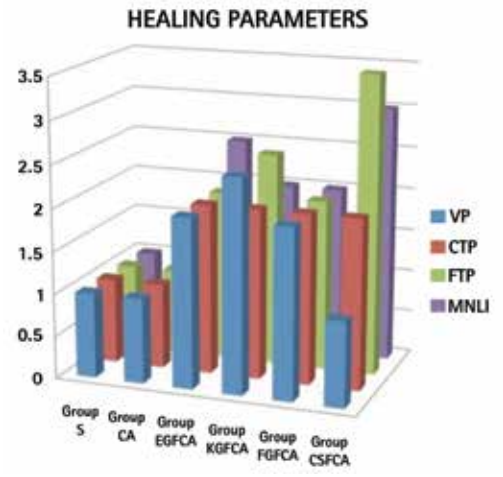



**Figure 3.** The distribution of histopathological scores of groups. The healing factors affecting anastomotic healing were presented seperately
VP: Vascular proliferation, CTP: collagene tissue proliferation, FTP: fibrous tissue proliferation, MNLI: mononuclear leukocyte infiltration, CA: colon anastomosis

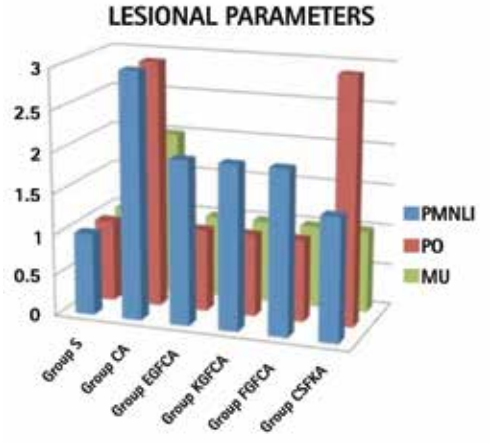



**Figure 4.** The distribution of histopathological scores of groups. The lesional factors affecting anastomotic healing were presented seperately
PMNLI: Polimorphonuclear leukocyte infiltration, MU: mucosal ulseration, CA: colon anastomosis

### Histopathologic Examination of the Anastomoses

Tissue samples of groups were analysed histopathologically by a single pathologist in a blinded fashion and scored in terms of healing parameters and lesional parameters (Figure 3, 4).

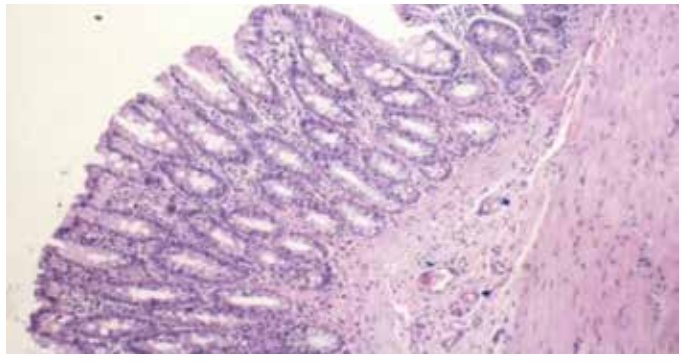
### Comparison of the Healing Results

**Sham Group Histopathological Examination (Picture 1):** Normal colonic mucosa.

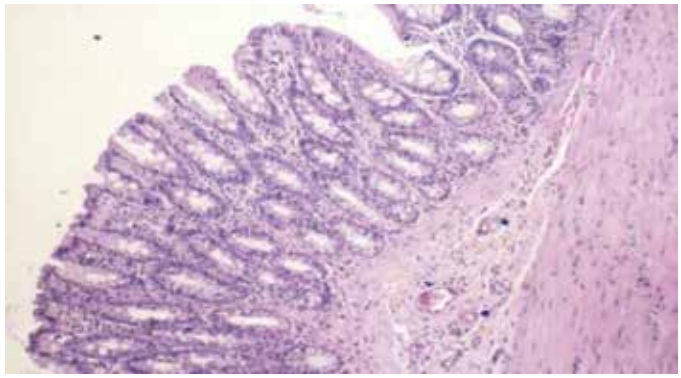
**Vascular Proliferation (Picture 2):** Comparison of groups from the point of VP revealed that only Group KGFCA scores were statistically higher than Group S ($p<0.001$). Comparison of colon anastomosis groups showed that only KGFCA groups scores were higher than Groups CA ($p<0.001$). There was no significant difference between Groups EGFCA, FGFCA, CSFCA and Group CA ($p>0.05$).

**Collagene Tissue Proliferation (Picture 3):** Although scores of all anastomosis groups were higher than Group S, there was no statistically significant difference between groups ($p>0.05$).

**Fibrous Tissue Proliferation (Picture 4):** Appraisal of groups in terms of FTP demonstrated that there was statistically significant difference between Groups FGFCA, KGFCA, CSFCA and Group S ($p<0.001$). Comparison of colon anastomosis groups indicated that there was significant



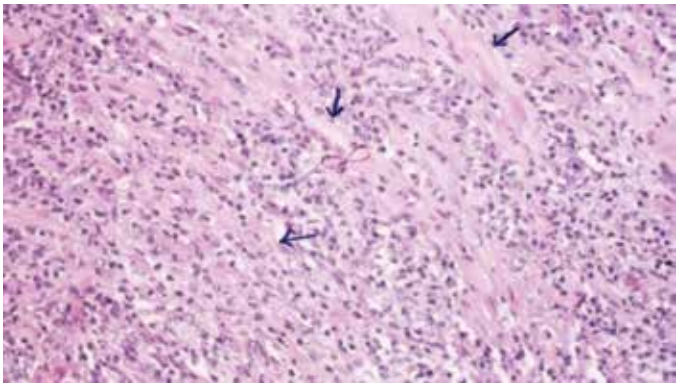
**Picture 1.** Normal colonic mucosa in Group S (HE, x100)



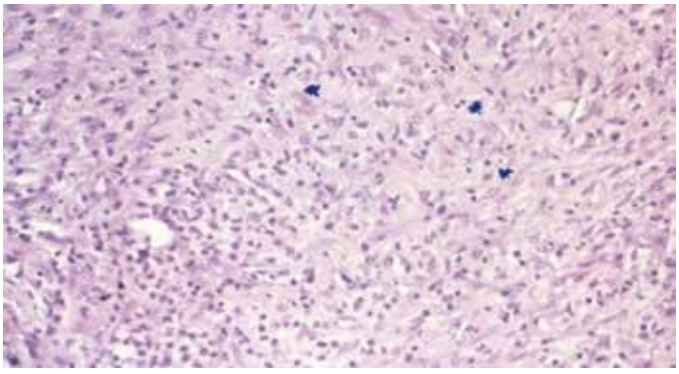
**Picture 2.** Vascular proliferation in Group keratinocyte growth factor colon anastomosis (HE, x100)

difference between Groups FGFCA, KGFCA, CSFCA and Group CA ($p<0.01$, $p<0.01$ and $p<0.001$, respectively). There was no difference between Group EGFCA and CA ($p>0.05$).

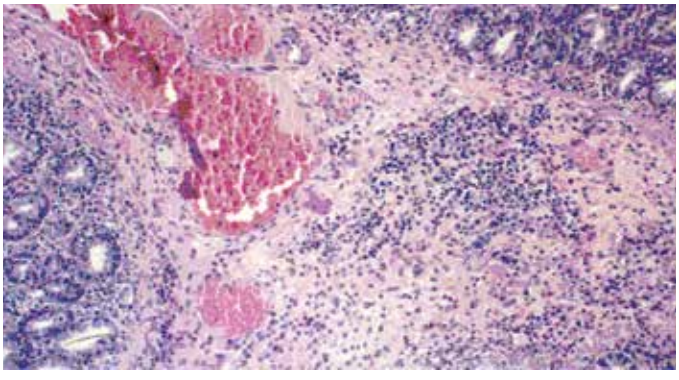
**Mononuclear Leukocyte Infiltration (Picture 5):** The MNLI scores of all groups were significantly higher than Group S ($p<0.001$). Comparison of colon anastomosis groups revealed that there was significant difference only between Group EGFCA and Group CA ($p<0.001$). There was no difference between Group KGFCA, FGFCA, CSFKA and Group CA ($p>0.05$).



**Picture 3.** Collagene tissue proliferation in Group epidermal growth factor colon anastomosis (HE, x200)



**Picture 4.** Fibrous tissue proliferation in Group keratinocyte growth factor colon anastomosis (HE, x200)

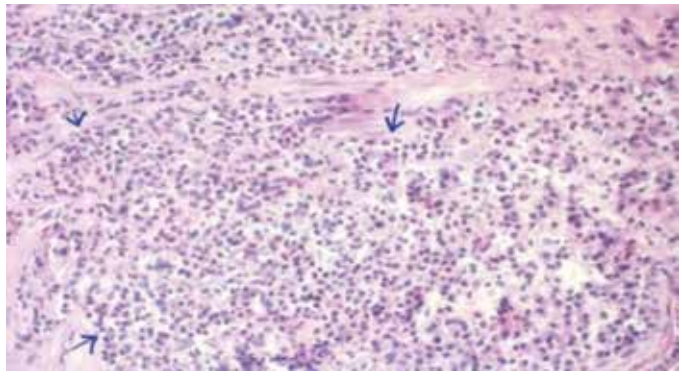


**Picture 5.** Mononuclear leukocyte infiltration in Group epidermal growth factor colon anastomosis (HE, x200)

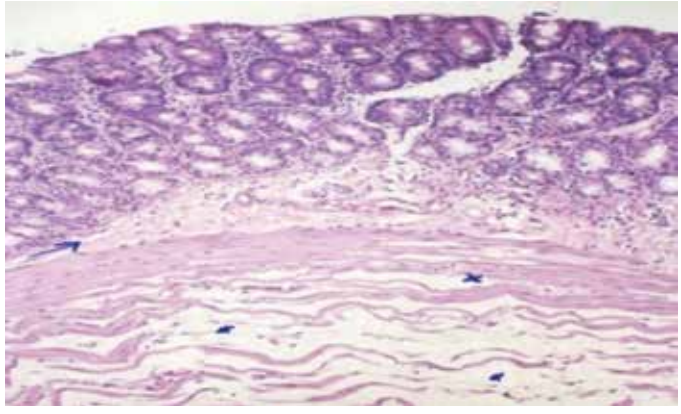
## Comparison of the Lesional Results

**Polimorphonuclear Leukocyte Infiltration (Picture 6):** The PMNLI scores of all groups were higher than scores of Group S ($p<0.001$). Analysis of colon anastomosis groups demonstrated the decrease of scores in Group EGFCA and FGFCA in comparison with Group CA ($p<0.001$). There was no significant difference between Group FGFCA, CSFCA and Group CA ($p>0.05$).

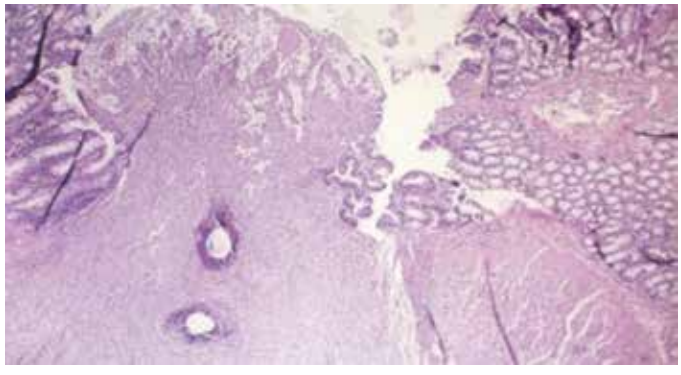
**Perianastomotic Oedema (Picture 7):** The scores of Group CA and CSFCA were higher than Group S ($p<0.001$); however there was no distinction between Group EGFCA,



**Picture 6.** Polimorphonuclear leukocyte infiltration in Grup colon anastamoses (HE, x200)



**Picture 7.** Perianastomotic oedema in Group colon anastamoses (HE, x100)



**Picture 8.** Mucosal ulseration in Grup CA (HE, x40)

FGFCA, KGFCA and Group S. The scores of Group EGFCA, FGFCA and KGFCA were better than scores of Group CA ($p<0.001$); there was no difference in comparison of group CSFCA and CA ($p>0.05$).

**Mucosal Ulseration (Picture 8):** The comparison of MU scores revealed that there was no difference between groups, although the scores of Groups EGFCA, FGFCA, KGFCA were better than Group S. There was no difference also between colon anastomosis groups ($p>0.05$).

## Discussion

Despite advances in technology and colorectal surgery, there is still high morbidity and mortality rates particularly due to anastomotic leakages.[1,2] The studies reported that when the anastomoses are closer to anus, leakage rates are higher.[2,25] Emergency colorectal surgery and attenuation of perianastomotic blood flow due to trauma or technical problems are the other important factors leading to anastomotic leaks.[1] Additionally, various factors like preoperative use of steroids, elongation of operation time and contamination of operation site increase the leaks rates.[3] Platell et al.[2] notify 2.4% overall leakage rate in a series of 1598 patients subjected to colorectal anastomoses while leakage rate is 1.5% in intraperitoneal anastomoses, the rate is 6.6% in extraperitoneal anastomoses.

Various physical and mechanical factors, such as different drug trials, examinations of angiogenetic mechanisms, a variety of colon cleaning methods, new suturing materials and surgical techniques were analysed in order to improve anastomotic safety.[26,27] Experimental study performed by Lord et al.[28] in order to compare colonic anastomoses performed with different sutures, such as chromic catgut, silk, polyglycolic acid, polypropylene, and teflon reported the eligibility of polypropylene and teflon sutures to cause less damage and inflammation. Chung[29] compared the single layer and double layer anastomoses and informed that double layer anastomoses reduce the mucosal blood flow. In accordance with literature, the anastomoses in present study were performed with single layer polypropylene.

In published studies mechanical strenght of anastomoses was measured via two principal methods: CBP was the more prefered, and other was longitudinal breaking strenght.[8,30] Biochemical method used for determination of the anastomotic healing in several studies was THP levels, and this measurement was accepted equal to collagene levels.[8]

Histopathology was also a beneficial assessment criterion in studies regarding to colonic anastomoses.[31] These leading parameters were used in our study.

Each GF affects on one or more different steps in wound healing process. These steps are inflammation, matrix synthesis and storage, angiogenesis, epithelization and contraction.[14,15] Recent studies research the effect of EGF on wound healing 18. EGF saturated sponges applied locally to colonic anastomoses, have shown to reverse the inhibitory effects of systemically applied methylprednisolone on wound healing.[32]

The appraisal of perianastomotic local EGF injection on colonic anastomoses revealed that EGF increases the CBP and THP levels and therefore the perianastomotic collagene levels. Additionally EGF has histopathologic benefits. MNLI has been significantly increased.

Although not singificantly, EGF increases other healing histopathologic parameters.

Furthermore the lesional parameters have been decreased. EGF significantly decreases the PMNLI and PAO. Although not significantly, the perianastomotic MU has been decreased.

The overall effects of EGF give chance to think that may be used in healing of colonic anastomoses. The insignificance of histopathologic parameters better than CA group, this may be explained with the insufficient number of experimental animals.

KGF is secreted by stromal cells of almost all epithelised organs and is mithogenic and chemotactic for epithelial cells.[15,33] Egger et al.[34] determined the beneficial effects of intraperitoneal EGF on colonic anastomoses and Cui et al. [35] on experimental esophagogastric anastomoses.

The appraisal of perianastomotic local injection of KGF in the present study revealed that; KGF increases the CBP, but not significantly and significantly increases the perianastomotic THP levels. Additionally histopathologic evaluation revealed the beneficial effects on VP and FTP. Although not significantly, other healing histopathologic parameters ameliorated. Moreover PAO decreased significantly. Other perianastomotic lesions diminished, but not significantly. These results showed that KGF is less effective than EGF and FGF on healing of colonic anastomoses. FGF is mithogenic for mesenchymal cells, stimulates angiogenesis and plays important role in wound healing.[20,36] Nurata et al. [37] in an experimental study, designed to investigate the effects of local FGF on duramather injuries with serebrospinal fluid leaks, determined the favourable effects of FGF on healing of duramather. Ernst et al.[38] found that local administration of FGF accelerates the healing of gastric ulcers in experimental conditions. The assessment of animals subjected to perianastomotic local FGF injection revealed the following results: 1) the CBP and perianastomotic THP levels and accordingly the collagene amount were increased by FGF; 2) FGF has positive effects on histopathological

healing parameters. The FTP was significantly increased and although not significantly other healing parameters also have been increased; 3) additionally perianastomotic lesions have been reduced by FGF administration. There is significant decrease in PMNLI and PAO. Although not significantly the perianastomotic MU also have been decreased. All these detected effects occupies that FGF also may be used in healing of colonic anastomoses although less effectiveness to EGF. G-CSF stimulates the colony growth specific to granulocytes and provides the functional activation of PMNLs.[39] Grzybowski et al.[40] have determined the favourable effects of locally administered G-CSF, GM-CSF and EGF on healing of skin incisions, but these findings were not confirmed by other similarly desinged studies. Present study showed that there was not significant increase in CBP and perianastomotic THP levels after injection of G-CSF. On histopathological point of view, among healing parameters only the FTP was significantly increased by G-CSF administration. Additionally there was not decrease in perianastomotic lesions. These effects demonstrate that G-CSF has not significant contribution on healing of colonic anastomoses.

Evaluation of all results determined in our study demonstrated that; G-CSF has not beneficial effects on wound healing of colonic anastomoses, but KGF, FGF, and particularly EGF are agents which may increase the anastomotic safety and resistance against the intracolonic tension strength of colonic anastomoses. This experimental trial is suggestive in terms of demonstrated that the local use of EGF, KGF, and FGF in colorectal surgery may provide benefits to surgeons, however most efficient GF need to be tested. The effectiveness and dose dependent benefits and the exact way of action and administration way of these drugs need to be investigated via further and detailed studies.

### Ethics

Ethics Committee Approval: DCR-D-10-00115.

Peer-review: Internally peer-reviewed.

### Authorship Contributions:

Surgical and Medical Practices: Ergin Kopal, Bartu Badak, Necdet Fatih Yaşar, Ersin Ateş, Concept: Ergin Kopal, Bartu Badak, Necdet Fatih Yaşar, Ersin Ateş, Design: Ergin Kopal, Ersin Ateş, Data Collection or Processing: Bartu Badak, Necdet Fatih Yaşar, Analysis or Interpretation: Ergin Kopal, Bartu Badak, Necdet Fatih Yaşar, Ersin Ateş, Literature Search: Bartu Badak, Necdet Fatih Yaşar, Writing: Bartu Badak.

Conflict of Interest: No conflict of interest was declared by the authors.

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## References

1. Buchs NC, Gervaz P, Secic M, Bucher P, Mugnier-Konrad B, Morel P. Incidence, consequences, and risk factors for anastomotic dehiscence after colorectal surgery: a prospective monocentric study. Int J Colorectal Dis 2008;23:265-270.
2. Platell C, Barwood N, Dorfmann G, Makin G. The incidence of anastomotic leaks in patients undergoing colorectal surgery. Colorectal Dis 2007;9:71-79.
3. Konishi T, Watanabe T, Kishimoto J, Nagawa H. Risk factors for anastomotic leakage after surgery for colorectal cancer: results of prospective surveillance. J Am Coll Surg 2006;202:439-444.
4. Senagore A, Milsom JW, Walshaw RK, Dunstan R, Chaudry IH. Does a proximal colostomy affect colorectal anastomotic healing? Dis Colon Rectum 1992;35:182-188.
5. Shikata J, Shida T, Satoh S, Furuya K, Kamiyama A. The effect of local blood flow on the healing of experimental intestinal anastomoses. Surg Gynecol Obstet 1982;154:657-661.
6. Foster ME, Laycock JR, Silver IA, Leaper DJ. Hypovolaemia and healing in colonic anastomoses. Br J Surg 1985;72:831-834.
7. Kashiwagi H. The lower limit of tissue blood flow for safe colonic anastomosis: an experimental study using laser Doppler velocimetry. Surg Today 1993;23:430-438.
8. Höckel M, Schlenger K, Doctrow S, Kissel T, Vaupel P. Theropeutic angiogenesis. Arc Surg 1993;128:423-429.
9. Jiborn H, Ahonen J, Zederfeldt B. Healing of experimental colonic anastomoses. III. Collagen metabolism in the colon after left colon resection. Am J Surg 1980;139:398-405.
10. Oxlund H, Christensen H, Seyer-Hansen M, Andreassen TT. Collagen deposition and mechanical strength of colon anastomoses and skin incisional wounds of rats. J Surg Res 1996;66:25-30.
11. Savage FJ, Lacombe DL, Boulos PB, Hembry RM. Role of matrix metalloproteinases in healing of colonic anastomosis. Dis Colon Rectum 1997;40:962-970.
12. Mastboom WJ, Hendriks T, de Boer HH. Collagen changes around intestinal anastomoses in germ-free rats. Br J Surg 1989;76:797-801.
13. Syk I, Agren MS, Adawi D, Jeppsson B. Inhibition of matrix metalloproteinases enhances breaking strength of colonic anastomoses in an experimental model. Br J Surg 2001;88:228-234.
14. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M. Growth factors and cytokines in wound healing. Wound Repair Regen 2008;16:585-601.
15. Komarcevic A. The modern approach to wound treatment. Med Pregl 2000;53:363-368.
16. DiBiase MD, Rhodes CT. The design of analytical methods for use in topical epidermal growth factor product development. J Pharm Pharmacol 1991;43:553-558.
17. Bazley LA, Gullick WJ. The eidermal growth factor receptor family. Endocr Relat Cancer 2005;1751:110-117.
18. Fisher DA, Lakshmanan J. Metabolism and effects of epidermal growth factor and related growth factors in mammals. Endocr Rev 1990;11;418-442.
19. Davidson D, Blanc A, Filion D, Wang H, Plut P, Pfeffer G, Buschmann MD, Henderson JE. Fibroblast growth factor (FGF) 18 signals through FGF receptor 3 to promote chondrogenesis. J Biol Chem 2005;280:20509-20515.
20. Szlachcic A, Zakrzewska M, Krowarsch D, Os V, Helland R, Smalås AO, Otlewski J. Structure of a highly stable mutant of human fibroblast growth factor 1. Acta Crystallogr D Biol Crystallogr 2009;65:67-73.
21. Motomura K, Hagiwara A, Komi-Kuramochi A, Hanyu Y, Honda E, Suzuki M, Kimura M, Oki J, Asada M, Sakaguchi N, Nakayama F, Akashi M, Imamura T. An FGF1:FGF2 chimeric growth factor exhibits universal FGF receptor specificity, enhanced stability and augmented activity useful for epithelial proliferation and radioprotection. Biochim Biophys Acta 2008;1780:1432-1440.

22. Rubin JS, Bottaro DP, Chedid M, Miki T, Ron D, Cheon G, Taylor WG, Fortney E, Sakata H, Finch PW, et al. Keratinocyte growth factor. Cell Biol Int 1995;19:399-411.
23. Basilico C, Moscatelli D. The FGF family of growth factors and oncogenes. Adv Cancer Res 1992;59:115-165.
24. Thomas J, Liu F, Link DC. Mechanisms of mobilization of hematopoietic progenitors with granulocyte colony-stimulating factor. Curr Opin Hematol 2002;9:183-189.
25. Martel G, Al-Suhaibani Y, Moloo H, Haggar F, Friedlich M, Mamazza J, Poulin EC, Stern H, Boushey RP. Neoadjuvant therapy and anastomotic leak after tumor-specific mesorectal excision for rectal cancer. Dis Colon Rectum 2008;51:1195-1201.
26. Ballantyne GH. The experimental basis of intestinal suturing. Effect of surgical technique, inflammation and infection on enteric wound healing. Dis Colon Rectum 1984;27:61-71.
27. Milsom JW, Senagore A, Walshaw RK, Mostosky UV, Wang P, Johnson W, Chaudry IH. Preoperative radiation therapy produces an early and persistent reduction in colorectal anastomotic blood flow. J Surg Res 1992;53:464-469.
28. Lord MG, Broughton AC, Williams HT. A morphometric study on the effect of suturing the submucosa of the large intestine. Surg Gynecol Obstet 1978;146:211-216.
29. Chung RS. Blood flow in colonic anastomoses. Effect of stapling and suturing. Ann Surg 1987;206:335-339.
30. Dignass AU, Tsunekawa S, Podolsky DK. Fibroblast growth factors modulate intestinal epithelial cell growth and migration. Gastroenterology 1994;106:1254-1262.
31. Hendriks T, Vereecken TH, Hesp WL, Schillings PH, de Boer HH. Loss of collagen from experimental intestinal anastomoses: early events. Exp Mol Pathol 1985;42:411-418.
32. Sakallioglu AE, Yagmurlu A, Dindar H, Hasirci N, Renda N, Deveci MS. Sustained local application of low-dose epidermal growth factor on steroid-inhibited colonic wound healing. J Pediatr Surg 2004;39:591-595.
33. Jimenez PA, Rampy MA. Keratinocyte growth factor-2 accelerates wound healing in incisional wounds. J Surg Res 1999;81:238-242.
34. Egger B, Tolmos J, Procaccino F, Sarosi I, Friess H, Büchler MW, Stamos M, Eysselein VE. Keratinocyte growth factor promotes healing of left-sided colon anastomoses. Am J Surg 1998;176:18-24.
35. Cui Y, Urschel JD, Petrelli NJ. The effect of keratinocyte growth factor-2 on esophagogastric anastomotic wound healing in rats. Int J Surg Investig 1999;1:307-309.
36. Cheng B, Fu X, Sheng Z, Gu X, Sun T, Sun X. The effect of basic fibroblast growth factor on myofibroblasts and its significance on wound healing. Zhonghua Yi Xue Za Zhi 2002;82:1187-1191.
37. Nurata H, Cemil B, Kurt G, Uçankuş NL, Dogulu F, Omeroğlu S. The role of fibroblast growth factor-2 in healing the dura mater after inducing cerebrospinal fluid leakage in rats. J Clin Neurosci 2009;16:542-544.
38. Ernst H, Konturek PC, Hahn EG, Stosiek HP, Brzozowski T, Konturek SJ. Effect of local injection with basic fibroblast growth factor (BFGF) and neutralizing antibody to BFGF on gastric ulcer healing, gastric secretion, angiogenesis and gastric blood flow. J Physiol Pharmacol 2001;52:377-390.
39. Souza LM, Boone TC, Gabrilove J, Lai PH, Zsebo KM, Murdock DC, Chazin VR, Bruszewski J, Lu H, Chen KK, et al. Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. Science 1986;232:61-65.
40. Grzybowski J, Otdak E, Janiak MK. Local application of G-CSF, GM-CSF and EGF in treatment of wounds. Postepy Hig Med Dosw 1999;53:75-86.