Epidermal büyüme faktörünün anastomoz, fasya ve cilt yara iyileşmesi üzerine etkisi

The effect of epidermal growth factor on anastomosis, fascia, and skin wound healing

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AMAÇ
Bu çalışmada amacımız epidermal büyüme faktörünün (EGF) ratlarda oluşturulan gastrojejunostomi anastomozunun fasya ve cilt yaralarının iyileşmesi üzerine etkisini araştırmaktı.

GEREÇ VE YÖNTEM
Hayvanlar (ratlar) iki ana gruba ayrıldı. Birinci gruptaki (kontrol grubu n:23) ratlar EGF verilmeksizin standart diyet ve musluk suyu ile beslendi. İkinci grupta (EGF grubu n: 19) deneklerin diyetine EGF eklendi. Tüm deneklerde laparotomi ve gastrojejunostomi aracılığı ile cilt fasya ve anastomoz olmak üzere üç değişik alanda yara oluşturuldu. Her iki grupta ratlar üç altgruba ayrıldı ve operasyondan sonra 3., 7. ve 21. günlerde denekler feda edildi. Operasyon sonrası 3., 7. ve 21. günlerde her iki grupta yara iyileşmesini değerlendirmek amacıyla deri ve fasya gerilme kuvveti ve de anastomoz patlama basıncı ölçüldü.

BULGULAR
EGF grubunda anastomoz gerilme kuvvetinin ameliyat sonrası 3. günde kontrol grubundaki anastomoz gerilme kuvvetinden kayda değer şekilde daha yüksek olduğu saptandı ve anastomoz patlama basıncı ve fasya gerilme kuvveti ve de cilt gerilme kuvveti her iki grupta birbirleri ile karşılaştırıldığında aralarında kayda değer bir fark saptanmadı.

SONUÇ
Girişimin 3. gününde epidermal büyüme faktörü verilen grubun gastrojejunostomi alanında daha belirgin fibroblastik hareketlenme olduğu ve sözü edilen günde sadece anastomoz kopma kuvvetinin kontrol grubuna göre daha üstün olduğu yapılan ölçümlerle gösterildi.

Anahtar sözcükler: Epidermal büyüme faktörü, anastomoz, yara iyileşmesi

BACKGROUND
The effect of epidermal growth factor (EGF) on gastrojejunostomy (anastomosis), fascia, and skin wound healing in rats was investigated.

METHODS
The animals (rats) were separated into two main groups. In the first group (control group n:23), rats were fed on standard diet and tap water without administration of EGF. In the second group (EGF group, n:19), EGF was added to the diet. Skin, fascia, and anastomosis wounds were created on three different locations via laparotomy and gastrojejunostomy in all rats. In both groups, the rats divided into three subgroups were sacrificed on the 3rd, 7th, and 21st days post operatively. Tensile strength of skin and fascia and bursting pressure strength were measured for wound healing in both groups on 3rd, 7th, and 21st days.

RESULTS
Anastomosis tensile strength of EGF group on the 3rd day of postoperation was found to be significantly higher than that of the control group and when we compared anastomosis bursting pressure, fascia tensile strength, and skin tensile strength in both groups, we did not find any significant differences.

CONCLUSION
The measurements indicated that on the 3rd day EGF administered group, which had a more remarkable fibroblastic activity at gastrojejunostomy site, was superior to the control group only in terms of anastomosis breaking tensile strength.

Key words: EGF, anastomosis, wound healing

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INTRODUCTION

Wound healing stages consist of inflammation, synthesis and maturation of collagen, fibroblast activity, neovascularization and epithelial migration. One of the numerous growth factors that organize the new cell and tissue formation via nutrients that are necessary for wound healing such as protein, carbonhydrate, lipid, mineral and vitamin is epidermal growth factor (EGF).[1-3] It has been demonstrated that systemic administration of EGF accelerates wound healing by reducing the degree of gastrointestinal destruction.[4,5]

It has also been demonstrated that EGF stimulates epidermal keratinocytes proliferation, [6] dermal fibroblast proliferation, [7] and epidermal regeneration [8] also wound healing by stimulating the synthesis of extracellular matrix protein such as collagen and fibronectin.[9] Moreover, it increases the production of other growth factors and their effects on cells.[10,11]

Wound healing takes place during the first 7-14 days at gastrojejunostomy sites. Both gastric acid content and pancreatic and biliary secretions threaten the anastomosis. If the amount of collagen is minimal at the wound area, they also increase the risk of leakage that is usually seen between 3-5 days after the operation.

In this study, we aimed to investigate the effect of EGF on gastrojejunostomy, abdominal wall, and skin wound.

MATERIALS AND METHODS

Forty-two rats (14-16 weeks of age, 250-300 gr, Spraque-Dawley) were used in this study. The animals, each one in a different cage in air-conditioned room at 21°C under a 12-hour lighting cycle, received a standard maintenance diet. The animals were separated into two main groups. In the first group (control group, n=23), rats were fed on standard diet and tap water. In the second group (EGF group, n=19), EGF was added to the diet. Skin, fascia, and anastomosis wounds were created on three different locations via laparotomy and gastrojejunostomy in all rats. In both groups the rats were divided into three subgroups to be sacrificed on the 3rd,7th and 21st days after the operation.

No food and water were given to the rats for 3-4 hours before the operation. Postoperatively, the animals received only water on the first, Ensure-Plus, on the second, and standard diet on the third day.

EGF: EGF (Sigma Chemical Company, USA) was mixed with 0.9% NaCl and kept at 4°C. In order to start the mitotic effect on cells (to leave behind the refractory period), EGF was administered into the gastric submucosa during the operation, and intraperitoneally 8.16.24. and 36. hours postoperatively at a dose of 10 mg/kg using 29 G needle, respectively. Operations and histopathologic examinations were performed by the same operator and the pathologist.

Surgical technique: The animals were anesthetized by intraperitoneal administration of 100 mg/kg ketamine + 30 mg/kg chlorpromazine. During the induction, a single dose of 50 mg/kg intramuscular ceftriaxon was administered. Following a 3 cm-midline incision, starting from the xyphoid, upper part of the jejunum at a distance of 5 cm from the Treitz and antrum were anastomosed. The abdominal wall was then closed continuously in two layers by using 5.0 vicryl and the skin was closed with interrupted 3.0 silk sutures.

After the experimental period all the animals were sacrificed with an overdose of ether. The entire incision line, including surrounding intact skin and fascia was excised. Macroscopic evaluation of intraabdominal adhesions, leakage at the anastomosis site, abscess formation and peritonitis was performed. The esophagus, stomach, duodenum and jejunum 5 cm distal to the anastomosis were cut and removed *en bloc*. Samples, 1 cm in width and 4 cm in length, were taken from the fascia and skin on the incision for biomechanical and histopathological studies.

Biomechanical studies: Biomechanical studies were conducted within an hour after the removal of tissues. The rats which had shown leakage at the anastomosis site, abscess formation, and peritonitis were excluded from the studies. Prior to the test, the specimens were preserved in saline and the samples taken for the histopathologic study were preserved in 10% formaline. Before the measurement of the fascia breaking strength, the uninterrupted sutures applied at the abdominal closure were cut off at the incision lateral without damaging the wound, but the sutures were not removed. The skin sutures were removed before the measurement of the skin tensile strength. Anastomosis, fascia, and skin tensile strengths

Table 1. The mean values of anastomosis bursting pressures, anastomosis tensile strengths, fascia tensile strengths, skin tensile strengths 3rd, 7th, 21st days of the experiment and statistical results.

Postoperative Day	EGF group (mean ± SD)			Control group (mean ± SD)				Statistical results			
	ABP	ATS	FTS	STS	ABP	ATS	FTS	STS	ABP	ATS	FTS	STS
3. day	40±12.7	100±20.3	84±11.9	35±13.6	30±7.9	73±16.0	80±13.6	40±9.3	Z=-1.38, p=0.16	Z=-2.00, p=0.04#	Z=0-0.63, p=0.52	Z=-52, p=0.59
7.day	150±40.3	190±34.0	220±29.3	160±21.7	135±23.4	180±28.9	200±29.7	140±25.4	Z=-0.73, p=0.46	Z=-0.063, p=0.52	Z=-0.83, p=0.40	Z=-1.04, p=0.29
21.day	280±47.8	380±83.7	660±77.4	546±65.8	280±41.3	300±74.4	648±100.8	544±92.6	Z=-0.62, p=0.52	Z=-1.25, p=0.20	Z=-0.20, p=0.83	Z=-0.41, p=0.67

EGF: Epidermal growth factor, SD: Standart deviation, ABP: Anastomosis bursting pressure, ATS: Anastomosis tensile strength, FTS: Fascia tensile strength, STS: Skin tensile strength, # Significant.

were tested in a specially constructed tensinometer which provided a constantly increasing force. The anastomosis, skin, and fascia samples were fixed with a clamp at one end and another clamp at the other end to which a tensinometer was attached. To operate this tensinometer, water was poured from a height of 50 cm with a speed of 60cc/min and tensile strength value (weigth) measured at the moment of breaking. To measure anastomosis bursting pressure polyethylene cannula, with a diameter of 1.5 mm, fastened to a Riester aneroid manometer apparatus was placed in the oesophagus and fastened around the oesophagus with 3.0 silk sutures. The duodenum and both afferent and efferent loops were ligated with 3.0 silk sutures, the former 1 cm away from the pylorus and the latter 3 cm away from the anastomosis. The pressure was gradually increased in a transparent glass jar containing saline at a heigth of 12 cm and the pressure value was recorded as the anastomosis bursting pressure when an air leakage occured at the anastomosis area. In order to demonstrate the anastomosis tensile strength, clamps were placed parallel to and 1 cm above the anastomosis. The area separated from the mesentery at afferent and efferent loops was ligated with 3.0 silk sutures and they were fixed with clamps 3 cm apart from the anastomosis. In addition, the tensinometer was connected to both clamps. The value measured on the special tensinometer at the moment of anastomosis breakage was recorded as the anastomosis breaking strength.

After the biomechanical studies, the anastomosis area was cut at a point of 1 cm away from the anastomosis line and preserved in 10% formalin for histopathologic investigation.

Mann-Whitney U and Fisher's Exact Test were used for statistical analysis.

RESULTS

In both groups, the skin and fascia tensile strengths, the mean values of anastomosis bursting and tensile strengths on the 3rd, 7th and 21st days of the experiment, and the statistical comparisons of the groups are shown in Table 1

Anastomosis leakage in gastrojejunostomy has been observed in four animals of the control group and in two of the EGF group, which also died on the 21st day of the experiment.

Three animals in the control group and one in the EGF group had the evidence of peritonitis. No significant increase in adhesion formation in the abdomen except liver adhesions to the anastomosis site was observed in both groups except the ones with peritonitis and leakage at the anastomosis site. When compared with the EGF administered groups, no significant difference was found in terms of leakage at the anastomosis site, intraabdominal abscess, and peritonitis. Four animals of the EGF group and eight animals of the control group showing leakage at the anastomosis site, intraabdominal abscess, and peritonitis were not included in the biomechanical studies.

Third day histology: Fibrin on gastrojejunostomy site, neovascularization and inflammatory response were observed in both groups; however, fibroblastic activity was more perceptible in the EGF administered group.

Granulation tissue formation at the skin sectional areas, presence of fibrin, neovascularization, and activity of normal fibroblasts were also evaluated. Formation of granulation tissue, neovascularization, inflamed cell infiltration, and weak fibroblast activity were observed in the fascial specimens of both groups.

Seventh day histology: The fibroblastic acti-

vity at the anastomosis site was (++) for the control group but it was (+++) for the EGF administered group. The synthesis of collagen was considered to be unsatisfactory in both groups.

The existence of skin collagen was detected to be (++) for the control group and (+++) for the EGF administered group. At fascia, organization of the granulation tissue was observed in the EGF administered group, yet the recession of fibrin and inflammatory reaction was seen to be incomplete in the control group.

21st day histology: It has been observed that concentration of the collagen was (++) in both groups and the granulation tissue was well organized at gastrojejunostomy sites. In both groups granulation tissue formed, and collagen existed at the skin. However, only EGF administered group was seen to have complete cicatrization. On the other hand, both groups had shown complete granulation tissue formation at fascia.

DISCUSSION

Recent studies have shown that wound healing, which is a complex biological process, develops as a result of autocrine and panacrine factors acting alone or together.[9] The existence of protein, lipid, carbonhydrate, vitamin and minerals, which are needed for extracellular matrix and new cell synthesis in the environment does not solely start the process of new cell synthesis. Some tissue developing factors such as EGF must also exist in the environment for the formation and regulation of new cell synthesis and extracellular matrix formation.[9]

It has been shown that EGF promotes dose dependent cell division and neovascularization with mitotic effect,[10,12,13] accelerates epithelial cell migration [10,14] and the healing of experimental damage triggered in the gastrointestinal tract, [4] and increases tensile strength of incisions as demonstrated on the biomechanical strength tests.[1] Parenteral administration of EGF appears to be more effective in preventing drug-induced injury to the rat gastric mucosa than oragastric administration of the growth factor.[15] This is probably due to the rapid degradation of intact EGF to less active forms in the presence of acid and pepsin, as previously shown by Playford et al.[16] However, in a study conducted by Lugea et al, EGF was administered directly through a tube into the duodenal lumen, thus avoiding gastric digestion of the compound.[17] In our study, we proposed that the duodenal reflux due to the gastrojejunostomy would greatly reduce the gastric acidity and the composition of the gastrointestinal and duodenal contents at the anastomotic site..

Brown observed experimentally that a single dose of EGF administration was not enough to increase the tensile strength in rats with skin incisions, in comparison with the control group.[1] In our study, skin breaking strength values comply with those obtained by Brown.

Furthermore, Brown has shown that administration of a single dose of EGF, when combined with slowly released multilameller liposome (for about 5 days) increased the tensile strength by 200% in comparison with the control group on the 7th and 14th days.[1]

Celebi found in his study with rats that on the 15th day dermal wound breaking strength of the group which received EGF mixed with bioadhesive gel (0.2% Carbapol 940 polymer) was remarkably higher than those of the groups which had EGF in isotonic solution.[18] Skin breaking strength was not signifieantly different between EGF and the control group in our study.

In one of his studies, Cronin had investigated. the effects of the applied force after the 3rd day of the anastomosis bursting pressure measurements and found that it reached its peak value on the 7th and 10th days, and at the same time, the hydroxyproline concentration decreased 40% in accordance with the normal measurement at the first three days. However, on the 5th day it approached to the normal value, and on the 10th and 14th days it became higher than the normal.[19] Jonsson had shown that the newly performed anastomosis had about two thirds the strength of the nonoperative intestinal wall. During the first three days after surgery, anastomosis rapidly lost strength and on the third day the strength of anastomosis reduced to 15% of the immediate postoperative value, which caused a dramatic decrease in suture holding strength. From the fourth to the seventh day there was a rapid increase in anastomotic strength.[20] Kingsnorth reported that the continuous administration of EGF with intraperitoneal pump in pigs following linear incision of

the stomach, ileum, and colon caused a remarkable increase in the breaking strength 5 days after surgery, when compared with the control group. [21] In our experiment, measurements on the third day showed that the EGF group was superior to the control group only in terms of anastomosis breaking strength. But in terms of anastomosis bursting pressure, and fascia breaking strength no meaningful difference was observed. The difference found on the 3rd day became negligible on the 7th and 21st days.

It had been shown that there was a biochemical active region of 5-6 mm in length starting from the stomach-duodenum incision line and going into the healthy tissue.[22,23] A significant decrease in the collagen quantity one milimetre perimeter of the duodenal incision on the 5th postoperative day was noted , but no remarkable change was observed at tissues 6-7 mm away from the incision.[23] On the 7th day it was seen that there was a significant increase of the collagen concentration at the incision line and on the 10th day there was a remarkable increase of the collagen concentration at the stomach corpus wound in accordance with the collagen concentration of the intact tissue. On the 20th and 40th days, it was observed that the collagen concentration at the stomach and duodenum, and at both of the incisions still continued to increase.[23] In our study, when two groups were compared histopathologically, it was found that on the 3rd day the EGF administered group had a more remarkable fibroblastic activity at the gastrojejunostomy site. On the 7th day, although the fibroblastic activity was evaluated to be (++) at anastomosis in the control group, it was regarded as (+++) in the EGF administered group. On the other hand, at fascia, organization of granulation tissue was observed in the EGF administered group, whereas in the control group, it was seen that fibrin and inflammation infiltration recession was incomplete. On the 21st day, all histopathological parameters were observed to be similar in both groups.

In our study, evaluation of the operative findings revealed leakage at the anastomosis site in 4 rats in the control and 2 rats in the EGF administered group. Intraabdominal abscess was seen in 1 rat in the control and in 1 rat in the EGF administered group. Peritonitis was seen in 3 rats in the control and in 1 rat in the EGF administered group. In the view of these findings, there was no meaningful statistical difference between the two groups. However, the improvement brought by EGF at the anastomosis breaking strength combined with the existence of fibroblastic activity which was observed to be more durable in the EGF group in the histopathologic investigations may explain the reason for the excess of the anastomosis leakage in the control group.

CONCLUSION

We concluded that the administration of EGF to increase tensile strength at the anastomotic site can open new horizons in the concept of wound healing.

REFERENCES

1. Brown GL, Curtsinger LJ, White M, et al. Acceleration of tensile strength of incisions treated with EGF and TGF-b. Ann Surg 1988; 208:788-794.
2. Assoian RK, Grotendorst GR, Miller DM, et al. Cellular Transformation by coordinated action of three peptide growth factors from human platelets. Nature 1984; 308:804-806.
3. Postlethwaite AE, Keski-Oja J, Moses HL, et al. Stimulation of the chemotactic migration of human fibroblasts by transforming growth factor-b. J Exp Med 1987; 165:251-256.
4. Vinter-Jensen L. Pharmacological effects of epidermal growth factor (EGF) with focus on the urinary and gastrointestinal tracts. APMIS Suppl 1999; 93:1-42.
5. Goodlad RA, Wright NA. Epidermal growth factor (EGF). Baillieres Clin Gastroenterol 1996; 10(1):33-47.
6. Rheinwald JG, Green H. Epidermal growth factor and the multiplication of human epidermal keratinocytes. Nature 1977; 265:421-424.
7. Lembach KJ. Induction of human fibroblast proliferation by epidermal growth factor (EGF): Enhancement by an EGF-binding arginine esterase and by ascorbate. Proc Natl Acad Sci USA 1976; 73:183-187.
8. Brown GL, Curtsinger LJ, Brightwell JR, et al. Enhancement of epidermal regeneration by biosynthetic epidermal growth factor. J Exp Med 1986; 163:1319
9. Komarcevic A. The modern approach to wound teratment. Med Pregl 2000; 53(7-8):363-368.
10. Rudkin GH, Miller TA. Growth factor in surgery. Plastic and Reconstructive Surgery 1996; 97:469-476.

11. Bradley SJ, Garfinkle G, Walker E, et al. Increased expression of the epidermal growth factor receptor on human colon carcinoma cells. Arch Surg 1986; 121:1242-1247.

12. Roberts AB, Sporn MB, Assoian RK, et al. Transforming growth factor type beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. Proc Natl Acad Sci. USA 1986; 83(12):4167-4171.

13. Buckley A, Davidson JM, Kamerath CD, et al. Epidermal growth factor increases granulation tissue formation dose dependently. J Surg Res 1987; 43(4):322-328.

14. Nanney LB. Epidermal and dermal effects of epidermal growth factor during wound repair. J Invest Dermatol 1990; 94:624.

15. Romano M, Kraus ER, Boland CR, et al. Comparison between transforming growth factor alpha and epidermal growth factor in the protection of rat gastric mucosa against drug-induced injury. Ital J Gastroenterol 1994; 26:223-228.

16. Playford RJ, Marchbank T, Calnan DP, et al. Epidermal growth factor is digested to smaller, less active forms in acidic gastric juice. Gastroenterology 1995; 108:92-101.

17. Lugea A, Mourelle M, Domingo A, et al. Epidermal growth factor increases surface hydrophobicity and resistance to acid in the rat duodenum. Am J Physiol Gastrointest Liver Physiol 2001; 280(4):774-785.

18. Celebi N, Erden N, Gonul B, et al. Effects of epidermal growth factor dosage forms on dermal wound strength in mice. J Pharm Pharmacol 1994; 46(5):386-387.

19. Cronin K, Jackson DS, Dunphy JE. Changing bursting strength and collagen content of the healing colon. Surg Gynecol Obstet 1968; 126(4):747-753.

20. Jonsson K, Jiborn H, Zederfeldt B. Breaking strength of small intestinal anastomoses. Am J Surg 1983; 145(6):800-803.

21. Kingsnorth AN, Vowles R, Nash JR. Epidermal growth factor increases tensile strength in intestinal wounds in pigs. Br J Surg 1990; 77(4):409-412.

22. Gottrup F. Healing of incisional wounds in stomach and duodenum: influence of long – term healing on mechanical strength and collagen distribution. Acta Chir Scand 1983; 149(1):57-62.

23. Gottrup F. Healing of incisional wounds in stomach and duodenum. Collagen distribution and relation to mechanical strength. Am J Surg. 1981; 141(2):222-227.