

Effect of Topically Applied Charged Particles on Healing of Colonic Anastomoses

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Hypothesis: Various forms of electrical stimulation can improve wound healing in different tissues, but their application to gastrointestinal tract healing has not been investigated. We assumed that positively charged diethylaminoethyl cross-linked dextran bead (diethylaminoethyl Sephadex [DEAE-S]) particles would have a beneficial effect on the healing of colonic anastomoses.

Design: Experimental animal study.

Setting: Animal research laboratory of a university hospital.

Animals: Forty female Wistar albino rats.

Interventions: Right colonic transection and anastomosis was performed in 5 animal groups. The control group received no treatment; the placebo group, methylcellulose gel; and the DEAE-S group, DEAE-S in methyl cellulose gel applied topically around the anastomoses. The fecal peritonitis (FP) group underwent cecal ligation and perforation simultaneously with the anastomosis to cause FP; the FP+DEAE-S group also received DEAE-S applied around the anastomoses.

Main Outcome Measures: After the completion of

postoperative day 4, all rats were killed. Anastomotic bursting pressures and hydroxyproline concentrations in perianastomotic tissue were measured and compared.

Results: Mean bursting pressures were 115.1 mm Hg in the control group, 113.6 mm Hg in the placebo group, 159.4 mm Hg in the DEAE-S group, 62.8 mm Hg in the FP group, and 121.1 mm Hg in the FP+DEAE-S group ($P=.001$, 1-way analysis of variance [ANOVA]). The differences between the control vs DEAE-S groups, placebo vs DEAE-S groups, and FP vs FP+DEAE-S groups were significant ($P<.05$, t test). Mean hydroxyproline concentrations were 5.2 $\mu\text{g}/\text{mg}$ in the control group, 4.9 $\mu\text{g}/\text{mg}$ in the placebo group, 5.6 $\mu\text{g}/\text{mg}$ in the DEAE-S group, 4.5 $\mu\text{g}/\text{mg}$ in the FP group, and 5.4 $\mu\text{g}/\text{mg}$ in the FP+DEAE-S group ($P=.09$, 1-way ANOVA). The difference between the FP and FP+DEAE-S groups was significant ($P=.04$, t test).

Conclusions: A positively charged particle, DEAE-S, improves healing of colonic anastomoses in healthy rats and in rats with FP. This inexpensive, nontoxic material is easily applied and deserves further evaluation in gastrointestinal tract healing.

Arch Surg. 2002;137:813-817

GASTROINTESTINAL anastomoses are among the most frequently performed procedures in general surgery clinics. Numerous experimental and clinical studies in the literature have investigated the factors that have an effect on healing of gastrointestinal anastomoses. Although many local and systemic factors affecting anastomotic healing have been determined, anastomotic leaks still cause considerable morbidity and mortality in daily surgical practice. Therefore, research on systemically or locally applied materials that can improve anastomotic healing deserves attention.

The effect of electrical stimulation on wound healing was first realized in the 19th century; since then, the effects of electrical current and magnetic fields on healing of several tissues have been investigated. Although its role in clinical practice

has been limited, electrical stimulation was shown to have positive effects on wound healing.¹⁻¹⁰ Findings indicate that macrophage migration, fibroblast proliferation, and collagen synthesis may be enhanced by electrical stimulation. These effects are important steps in healing, but their mechanism is not clear.¹¹⁻¹⁴

In the past decade, apart from electrical currents and magnetic fields, charged particles were shown to have positive effects on the healing of skin wounds.¹⁵⁻¹⁹ Nevertheless, the effects of any form of electrical stimulation on the healing of gastrointestinal anastomoses have not been investigated adequately in the literature. The aim of this study was to evaluate the effect of diethylaminoethyl cross-linked dextran bead (diethylaminoethyl Sephadex [DEAE-S]; Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) particles, in the form of positively charged

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MATERIALS AND METHODS

Forty female Wistar albino rats weighing 200 to 250 g were used in the study. All animals received humane care according to the guidelines of the Animal Research Council of Hacettepe University, Ankara, Turkey, for the care and use of laboratory animals. All animals were allowed standard rat chow and water ad libitum. Twelve hours before anesthesia, animals were deprived of food but had free access to water. After the intramuscular induction of anesthesia with xylazine hydrochloride (Rompun), 5 mg/kg, and ketamine hydrochloride (Ketalar), 30 mg/kg, all rats underwent a midline laparotomy. The right colon was transected and an end-to-end anastomosis was performed using 1 layer of interrupted 6-0 polypropylene sutures (Prolene; Ethicon Ltd, Edinburgh, Scotland).

PREPARATION OF TEST PARTICLES

Ethylene oxide-sterilized methylcellulose powder (300 ctipoises) was dissolved in sterile saline solution using an aseptic technique, and 10% methylcellulose gel prepared in this manner was used as placebo. The DEAE-S (A25) beads were sterilized by means of ethylene oxide and suspended in sterile 10% methylcellulose gel. The concentration of the DEAE-S suspension was 20 mg/mL, and was used as test material. The placebo and test materials were prepared and homogenized 24 hours before surgery. These materials were highly viscous and sticky and preserved their form for days. These materials were applied inside the transection surfaces during performance of the anastomoses and around the suture line after completion of the anastomoses with the use of a 1-mL syringe without a needle.

ANIMAL GROUPS

Rats were divided into 5 groups, each including 8 animals. The control group underwent colonic transection and anastomosis, with no additional procedure until the animals were killed. During the colonic anastomosis procedure, the placebo group received 0.2 mL of 10% methylcellulose gel that was applied topically to transection surfaces and around the anastomosis. The DEAE-S group received 0.2 mL of DEAE-S suspended in 10% methylcellulose gel that was applied topically to transection surfaces and around the anastomosis during the colonic anastomosis procedure. The FP group underwent simultaneous cecal ligation and perforation during the colonic anastomosis

procedure to cause FP. The cecal wall was perforated proximally to the ligature only once with the tip of a No. 11 scalpel. The FP+DEAE-S group underwent simultaneous cecal ligation and perforation during the colonic anastomosis procedure to cause fecal peritonitis. During the colonic anastomosis procedure, 0.2 mL of DEAE-S suspended in 10% methylcellulose gel was applied topically to transection surfaces and around the anastomosis.

ANALYSIS OF ANASTOMOSES

After the procedures, the laparotomy wounds were closed with a double layer of continuous 4-0 silk sutures. Animals were fed with standard rodent chow and water ad libitum postoperatively. After the end of fourth postoperative day (100 ± 2 hours), all animals were killed by means of inhalation of a high dose of diethyl ether. After the animals were killed, relaparotomy was performed, the colonic anastomosis was dissected free of adhesions, and a 4-cm segment of colon with the anastomosis in the middle was resected. One end of this segment was closed with a ligature and a catheter was secured to the other end. Inside a glass jar filled with water, air was pumped into the segment of colon at a rate of 2 mL/min by means of an infusion pump. Intraluminal pressure was monitored while the air was pumped. The intraluminal pressure at which air leakage from the anastomosis occurred was recorded as the bursting pressure.^{20,21} This variable showed the mechanical strength of the anastomoses.

After the measurement of bursting pressure, a 1-cm segment of colon containing the anastomosis was resected to determine the hydroxyproline concentration in perianastomotic tissue, which represents the perianastomotic collagen concentration. Tissue hydroxyproline concentrations were determined by using the spectrophotometric method of Bergman and Loxley.²² Calculations were performed to express the results as micrograms of hydroxyproline per milligram of tissue.

The mean bursting pressures and tissue hydroxyproline concentrations of the groups were calculated and expressed as mean \pm SEM. The mean values of groups were compared by means of 1-way analysis of variance (ANOVA), and the groups were compared separately by unpaired, 2-tailed *t* test. Pearson correlation analysis was performed to define the correlation of bursting pressure and hydroxyproline concentration values of the animals. In these tests, $P < .05$ was considered statistically significant. We used SPSS for Windows, Version 8.0, software (SPSS Inc, Chicago, Ill) for statistical analysis.

beads, on the healing of colonic anastomoses. For this purpose, we applied DEAE-S particles topically around colonic anastomoses performed in healthy rats and rats with fecal peritonitis (FP) and evaluated variables of wound healing.

RESULTS

In this study, we evaluated anastomotic wound healing by means of 2 variables. One of these variables was bursting pressure, which showed the mechanical strength of the anastomoses. During the measurement of bursting pressures, all leaks occurred from the anastomoses. The difference be-

tween the groups was statistically significant according to the 1-way ANOVA ($P = .001$). The groups were compared one by one by the *t* test. The difference between the control and placebo groups was insignificant ($P = .9$), and the mean bursting pressure of the FP group was significantly lower than that of the control ($P = .006$) and placebo ($P = .01$) groups. The mean bursting pressure of the DEAE-S group was significantly higher than that of the control ($P = .04$) and placebo ($P = .04$) groups. Furthermore, the mean bursting pressure of the FP+DEAE-S group was significantly higher than that of the FP group ($P = .003$) and was nearly the same as that of the control ($P = .7$) and placebo ($P = .7$) groups (**Table** and **Figure 1**).

Bursting Pressure and Tissue Hydroxyproline Concentration Values of the Groups*

	Study Groups				
	Control	Placebo	DEAE-S	FP	FP + DEAE-S
Bursting pressure, mm Hg†	115.1 ± 13.9	113.6 ± 15.1	159.4 ± 16.1	62.8 ± 8.6	121.1 ± 13.6
Tissue HP concentration, µg/mg‡	5.2 ± 0.5	4.9 ± 0.3	5.6 ± 0.3	4.5 ± 0.2	5.4 ± 0.4

*Data are given as mean ± SEM. DEAE-S indicates diethylaminoethyl cross-linked dextran bead (diethylaminoethyl Sephadex); FP, fecal peritonitis; and HP, hydroxyproline. Groups are explained in the "Animal Groups" subsection of the "Materials and Methods" section.

† $P = .001$, 1-way analysis of variance (ANOVA).

‡ $P = .09$, 1-way ANOVA.

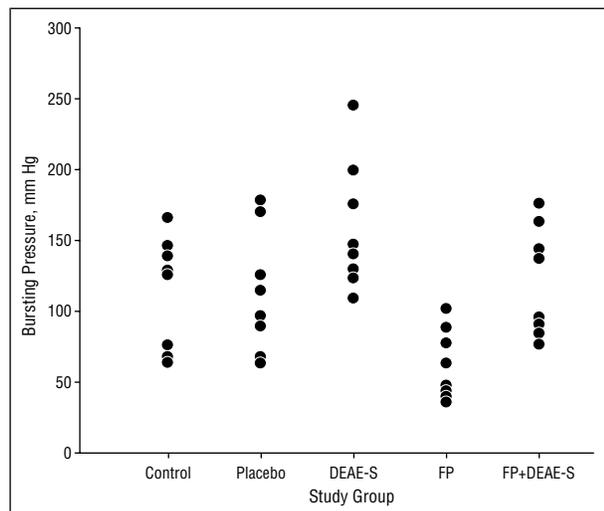


Figure 1. Bursting pressure values of the animal groups. DEAE-S indicates diethylaminoethyl Sephadex; FP, fecal peritonitis. Groups are described in the "Animal Groups" subsection of the "Materials and Methods" section.

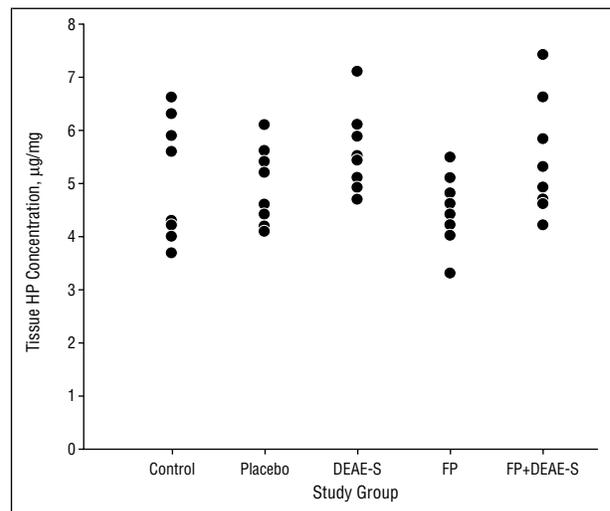


Figure 2. Tissue hydroxyproline (HP) concentration values of the animal groups. Other abbreviations are explained in the legend to Figure 1. Groups are described in the "Animal Groups" subsection of the "Materials and Methods" section.

The other variable used to evaluate anastomotic wound healing was the hydroxyproline concentration in perianastomotic tissue, which represented the collagen accumulation around the anastomoses. The hydroxyproline concentration values showed the same trend as the bursting pressure values, but the differences were small and did not reach statistical significance according to 1-way ANOVA ($P = .09$). Mean values of the control and placebo groups were nearly similar, and the mean value of the FP group was lower than these 2 groups. The DEAE-S group had higher hydroxyproline concentrations compared with the control and placebo groups. The mean value of the FP+DEAE-S group was higher than that of the FP group and slightly higher than those of the control and placebo groups. According to the *t* test, only the difference between the FP+DEAE-S and FP groups was statistically significant ($P = .04$) (Table and **Figure 2**).

The correlation of bursting pressures and tissue hydroxyproline concentrations of all animals was evaluated by Pearson correlation analysis. Both variables of wound healing had a positive good correlation ($P < .001$; $r = 0.71$) (**Figure 3**).

COMMENT

Gastrointestinal anastomoses are some of the most frequently performed procedures in surgery clinics. Fac-

tors affecting wound healing in anastomoses of the gastrointestinal system (GIS) have been investigated in numerous clinical and experimental studies, but anastomotic leaks continue to be the source of major morbidity. Despite the knowledge of many local and systemic factors affecting anastomotic healing, no widely used material or treatment in clinical practice reduces the rate of anastomotic leakage.

Electrical stimulation in the form of electrical currents, electrical fields, or topically applied charged particles has been shown to have beneficial effects on wound healing. Many clinical and experimental studies were performed in the second half of the 20th century to evaluate the effect of electrical stimulation on tissue healing and regeneration. In most of these studies, healing in the skin, subcutaneous tissues, and bone was investigated. Healing in GIS anastomoses associated with electrical stimulation is a topic that has not been investigated sufficiently in the literature. The aim of our study was to evaluate the effect of topically applied charged particles on the healing of GIS anastomoses in healthy rats and in rats with FP.

The main concept underlying the interaction of electrical stimulation and wound healing is the known occurrence of endogenous electrical fields and currents in injured tissue. The studies of healing incisions and bone injuries showed that an endogenous electrical potential is generated in the injured and healing tissues and is ter-

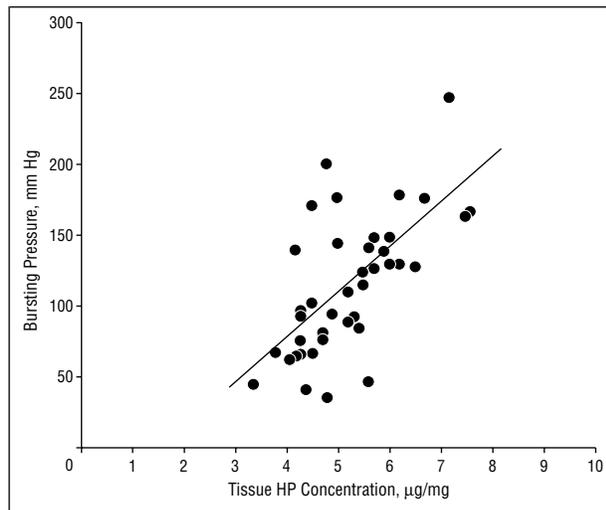


Figure 3. Bursting pressure values of the animals are plotted against tissue hydroxyproline (HP) concentration values, and linear curve estimation is shown ($P < .001$; $r = 0.71$). Other abbreviations are explained in the legend to Figure 1.

minated when healing is complete.²³⁻²⁶ Becker²⁵ theorized that injury to a living tissue causes a localized shift in current flow (current of injury) that triggers biological repair. The idea of reproducing and augmenting the endogenous electrical potential generated in injured and healing tissues by means of exogenous electrical stimulation formed the basis of studies trying to enhance wound healing by applying electrical currents or fields.

Although the exact mechanism is not known, different forms of electrical stimulation have been shown to improve wound healing clinically and experimentally. Experiments on amphibians showed that electrical stimulation improved limb regeneration in some species.^{25,27} Assimakopoulos¹ treated skin defects in rabbit ears by means of continuous direct current, which resulted in accelerated wound healing, thicker scars, and greater tensile strength compared with the control group. In similar studies, Konikoff² and Bigelow et al³ found skin incisions treated with continuous direct current to have increased tensile strength. Alvarez et al⁴ applied continuous direct current to partial-thickness wounds of pigs, and these wounds showed accelerated reepithelization and significantly increased dermal collagen production compared with controls. Taşkan et al⁵ showed an increased number of fibroblasts and increased collagen accumulation in electrically stimulated skin incisions in rats.

Some clinical studies on the topic of electrical stimulation and healing also have been performed. Electromagnetic fields have been used in clinical practice to improve healing of ununited fractures, and this stimulus was shown to trigger calcification of fibrocartilage in the gap between bone ends and to promote bony union.²⁸⁻³⁰ The studies in patients with chronic ischemic and decubitus ulcers proved that treatment with direct electrical current increased the proportion of completely healed ulcers and also accelerated the healing, compared with conventional wound therapy.⁶⁻¹⁰ However, the electrical stimulation used in these studies were not standard, and differences in application, time intervals, voltages, etc, were found. These differences limited the use of electri-

cal stimulation in daily clinical practice. Nevertheless, the positive effect of electrical stimulation on wound healing is clear. No adverse effects of electrotherapy exist. Furthermore, in electrically stimulated wounds, bacterial contamination is eliminated more easily, infection rates are lower, and less debridement is required.^{7,9,31,32}

The studies investigating the effects of electrical stimulation at the cellular level have supported the findings mentioned above. According to in vitro studies, electrical fields can increase migration, DNA synthesis, and collagen synthesis of fibroblasts.¹¹⁻¹³ Macrophages are known to have an important role in modulation of healing through the expression of some growth factors, and experimental studies showed that electrical charges can induce macrophage migration.¹⁴ Growth factors participate in the healing process, and transforming growth factor β plays a fundamental role in collagen synthesis.³³ Electrical stimulation increases the expression of receptors for transforming growth factor β on human dermal fibroblasts.³⁴

Another form of electrical stimulation consists of the application of charged particles. Implanted foreign bodies with a significant zeta potential have been suggested to mimic the bioelectric fields generated at wounds and, when the potential is high enough, to assist healing by affecting the cell cycle and altering cell behavior.³⁵ Topically applied charged particles may produce a fixed electrical field adjacent to the wound for a long time and it is sufficient to apply them once.¹⁵ These advantages led researchers to investigate the effects of charged particles on healing. Injection of charged particles to bone was observed to result in new intramedullary bone formation, and the most effective particles were DEAE-S beads.³⁶ Mustoe et al¹⁵ topically applied various charged beads to incisional wounds in rats and observed increased tensile strength with more cellular, collagen-rich dense connective tissue in wounds that were treated with DEAE-S beads compared with control wounds and wounds treated with other beads. Eppley et al^{17,38} injected DEAE-S beads in subcutaneous tissues of rats, which resulted in extensive macrophage infiltration without acute inflammation, followed by extensive intermaterial fibroblast and collagen ingrowth. No evidence of a foreign body or chronic inflammatory response was found, and the authors suggested that the beads have a chemotactic effect on macrophages and fibroblasts. In 7 other animal studies, topically applied DEAE-S beads were shown to improve healing and to increase the tensile strength of incisional wounds¹⁶⁻¹⁹; compared with other charged and uncharged beads, DEAE-S (especially A25) beads were superior in promoting wound healing.^{16,18,19} One of these studies, performed by Galiano et al,¹⁹ also proved the beneficial effect of DEAE-S beads in rats undergoing total-body irradiation and those undergoing surface irradiation; DEAE-S beads reversed the radiation-induced healing deficit and resulted in an increased tensile strength. The fate of these beads was evaluated histologically, and these particles were found to begin degenerating the second week.¹⁷

In our study, we evaluated the effect of DEAE-S, which had been proved to be the most effective charged particle in enhancing wound healing, on the healing of colonic anastomoses. We evaluated anastomotic healing in the early postoperative period, when most anastomotic leaks take

place. We used bursting pressure and hydroxyproline concentration values in perianastomotic tissue for comparisons, which are the most reliable and objective standard variables in evaluating anastomotic healing.^{20,21,39,40} Both variables had good correlation. Histological assessment was not used for comparisons, because the results are highly subjective and depend on the observer; it is not possible to achieve objective and reliable quantitative data by means of histological evaluation.^{39,40} Compared with healthy rats, we evaluated the use of DEAE-S in rats with FP. In clinical and experimental studies, intra-abdominal infection has definitely been shown to delay anastomotic healing.^{20,41-44} In our study, we used the development of FP to achieve a GIS anastomosis model with impaired healing, and according to our results, healing was significantly delayed in the FP group. Topical application of DEAE-S improved anastomotic healing compared with the control and placebo groups and reversed the healing deficit observed in rats with FP. Anastomotic healing reached the level of the control group in the FP + DEAE-S group.

Although the exact mechanisms are not clear, electrical stimulation seems to have a positive effect on wound healing. In an experimental setting, charged particles have the advantages of easy topical application, sufficiency of only 1 application, easy standardization, and low cost compared with other forms of electrical stimulation and other agents to promote healing. The DEAE-S beads were shown to improve the healing of bone, skin, and subcutaneous tissue in other studies, and we demonstrated the positive effect of DEAE-S beads on the healing of colonic anastomoses. In the clinical setting, additional treatment to improve healing may not be necessary in GIS anastomoses with a low risk for leakage. On the other hand, high-risk anastomoses performed in the presence of factors that delay wound healing may require additional treatment to decrease morbidity. When taken from this aspect, our findings indicate that DEAE-S deserves further investigation for use in clinical practice.

This study was presented at the International Surgical Week 2001, Brussels, Belgium, August 28, 2001.

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