Effect of Ascorbic Acid on Incisional Wound Healing in Streptozotocin-Induced Diabetic Rats

Erdinc Kamer, MD;1 Haluk Recai Unalp, MD;1 Omer Gundogan, MD;1 Gulden Diniz, MD;2 Ragip Ortac, MD;2 Murat Otuksan, MD;3 Hayrullah Derici, MD;1 Mehmet Ali Onal, MD1

WOUNDS 2010;22(2):27–31

From the 1Department of Surgery, Izmir Ataturk Training and Research Hospital, Izmir, Turkey; 2Department of Pathology, Dr. Behcet Uz Children’s Hospital, Izmir, Turkey; 3Department of Pharmacology, Ege University Faculty of Medicine, Bornova, Izmir, Turkey

Address correspondence to: Erdinc Kamer, MD
Department of Surgery
Izmir Ataturk Training and Research Hospital
1834 sk. No:9/4, 35530 Karsiyaka
Izmir, Turkey
Phone: +90 232 2444444/2546
E-mail: erdinc.kamer@gmail.com

Abstract: Background. Abdominal wall repair after celiotomy is important because insufficient incisional wound strength results in wound failures such as fascial dehiscence and herniation. Ascorbic acid has been shown to play an important role in wound healing. The purpose of this study was to investigate whether ascorbic acid improves incisional wound healing in a diabetic rat. Methods. Male Wistar-Albino streptozotocin-induced diabetic rats (n = 20) were divided into two groups: control group (CG; n = 10), and daily 200 mg/kg ascorbic acid (study group, [SG], n = 10) given orally. Ten animals from each group were euthanized on postoperative day (POD) 14 after wounding; breaking strength, histologic examination, and tissue hydroxyproline levels were analyzed. Results. The hydroxyproline tissue content of the abdominal fascia in the ascorbic acid treatment group was superior to the control group, and the difference was statistically significant (P < 0.05). The tensiometric analyses revealed that tensile strength for the midline incision was significantly higher in the study group compared to the control group (P < 0.05). Significant differences were found in the results of histologic examination of tissue specimens between the two groups regarding acute inflammation, chronic inflammation, granulation tissue fibroblast maturation, collagen deposition, and neovascularization on POD 14 (P < 0.05). Conclusion. The present study demonstrates that administration of ascorbic acid prior to laparotomy expedites wound healing in a rat. On the contrary, we suggest that it could confer benefits to tissue healing by significantly enhancing tissue hydroxyproline levels, neovascularization, fibroblast maturation, and collagen deposition.

H

ealing of the abdominal wall wound following celiotomy is important because insufficient incisional wound strength results in prolonged periods of disability for the patient secondary to fascial dehiscence and hernia formation.1 Wound healing is a natural restorative response to tissue injury. It is a complex and dynamic process with reconstitution and restoration of the tensile strength of injured skin or tissue.2 The normal healing process in healthy individuals takes place at an optimal rate but it is usually delayed or even impaired completely among patients with diabetes.3 This impaired wound healing in diabetes mellitus is a significant clinical problem.
High blood glucose hinders proliferation of cells and decreases collagen production. Furthermore, decreased chemotaxis and phagocytosis, a reduction in the levels of growth factors, and the inhibition of fibroblast proliferation have all been suggested to contribute to the observed impairment in wound healing. Ascorbic acid (vitamin C) has been shown in earlier investigations to have an unparalleled stimulatory effect on collagen-type synthesis. Ascorbic acid is an important modulator of collagen production and acts as a cofactor in hydroxylation of proline and lysine residues in procollagen. Ascorbic acid has been reported to increase collagen deposition and tensile strength in experimental models of wound repair. The role of ascorbic acid in the healing of injured tissues has been investigated in a variety of studies. However, effect of ascorbic acid on the incisional wound healing has not been studied in streptozotocin-induced diabetic rats. The purpose of this study was to investigate whether ascorbic acid improves incisional wound healing in a diabetic rat celiotomy model.

**Materials and Methods**

**Animals.** Twenty 5-month-old male Wistar-Albino rats at Ege University Faculty of Medicine animal research laboratory, weighing 250 g–300 g, were acclimated to the new environment for 48 hours; they were maintained on standard rat diet and water. They were fed standard laboratory diet until the night before the operation and had access to water ad libitum. All manipulations were undertaken in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the institutional animal ethical committee of Ege University Faculty of Medicine (Izmir, Turkey).

The 20 rats were randomized into two groups of 10 each. Control group (CG, n = 10) animals were fed standard laboratory diet and water ad libitum until 12 hours before surgery. Study group (SG, n = 10) were applied orally with orogastric tube 200 mg/kg once a day for 10 days of ascorbic acid (Sigma, Inc., St. Louis, MO) therapy until 12 hours before surgery. The ascorbic acid solution consisted of 100 mg/L ascorbic acid dissolved in 100 mL distilled water.

**Induction of diabetes mellitus.** Diabetes was induced by a single 65 mg/kg intravenous injection of streptozotocin (STZ; Sigma, Inc.), a toxin specific for insulin-producing cells, in saline-sodium citrate buffer ([pH 4.5] Sigma, Inc.). Blood glucose levels were measured using a Glucostix (Bayer, Istanbul, Turkey) and a glucometer (Glucometre II Model 5550, Ames, IN). Ten days after STZ injection, animals with blood glucose level above 300 mg/dL were defined as diabetic and used for study purposes.

**Operative procedure.** Each rat was anesthetized with an intramuscular injection of 60 mg/kg of ketamine hydrochloride (Ketalar, Eczacibasi, Warner-Lambert Laboratories, Levent, Istanbul, Turkey) and 10 mg/kg of xylazine hydrochloride (Rompun, Bayer Laboratories, Sisli, Istanbul, Turkey). All procedures were performed under clean, but nonsterile conditions. A 4-cm midline laparotomy was performed after the abdominal skin was shaved with a povidone-iodine scrub. Next, the abdominal fascia and skin were closed in a continuous fashion with running 3/0 silk sutures. All rats were given water and a regular diet ad libitum on the day of the operation.

Ten animals in each group were sacrificed at POD 14 with an overdose of sodium pentobarbital (300 mg/kg intraperitoneal). The skin sutures were removed and tensile strength of the midline incision was measured (mmHg) using a tensiometer as described by Gulcelik et al. Two surgeons, who were blinded to the groups, performed the measurements. Pressure that caused the incision line to separate was defined as the tensile strength. After the assessment of the tensile strength analysis had been completed, the entire incision line including surrounding intact skin and fascia (1 cm wide) was excised. The abdominal incision wounds were excised and divided into two pieces (2 cm x 1 cm) in all animals. One piece was fixed into a 10% formaldehyde solution and stored for pathologic examinations. The second piece was used to measure hydroxyproline levels and was examined histologically. Two pathologists examined all representative fascia sections in each rat histologically under a light microscope in blinded fashion.

**Tensile strength.** The skin sutures were removed and tensile strength of the midline incision was measured (mmHg) using a tensiometer as described by Ekiz et al. This analysis was conducted within 1 hour after the removal of tissue. Before the measurement of the fascia tensile strength, the uninterrupted sutures applied at the abdominal closure were cut off at the incision lateral without damaging the wound. Wound samples were secured with a clamp at one end and another clamp at the other end to which a tensiometer was attached. Water was poured from a height of 50 cm at a rate of 60 cc/min; the tensile strength value (weight) was measured at the moment of breaking. A small piece of the incision just

**Histologic grading.** A small piece of the incision just

---

Kamer et al
superior to the sample used to assess rupture strength was processed for routine histologic examination. Biopsy specimens from each fascial wound were obtained as described above on POD 14. At each time point, the wounds were harvested and their histologic features were assessed in paraffin-embedded sections using hematoxylin and eosin (H&E) and Gomori’s trichrome stains at a magnification of x100–x400. The main histologic outcome measures included the amount of acute and chronic inflammatory infiltrates, the amount and maturation of granulation tissue, collagen deposition, reepithelialization, and neovascularization. The Abramov’s histologic scoring system was used for this study.9 Abramov’s system assessed each parameter independently and assigned a score of 0–3. Acute and chronic inflammatory infiltrates, the amount of granulation tissue, and collagen deposition were graded as: 0 (none), 1 (scant), 2 (moderate), 3 (abundant). The fibroblast maturation of granulation tissue was graded as: 0 (immature), 1 (mild maturation), 2 (moderate maturation), 3 (fully matured). Neovascularization was graded as: 0 (none), 1 (up to five vessels per high-power field [HPF]), 2 (6–10 vessels per HPF), 3 (more than 10 vessels per HPF).7 Two independent pathologists performed the histological examination and applied the scoring system in a blinded fashion.

**Hydroxyproline analyzed.** Another 2-cm x 1-cm portion of the abdominal wall sample, including the suture line in the middle, was frozen in liquid nitrogen and stored at -80˚C for further biochemical analysis. After the samples had been thawed, dried, weighed, and homogenized separately, the hydroxyproline contents were determined according to the method of Prochop and Kivirikko mg/100 g of tissue.10

### Statistical Analysis

The results were expressed as mean ± SD. Mann-Whitney U test was used for statistical analysis. Inter- and intraobserver variabilities were calculated using the Cohen’s κ test. P < 0.05 was considered statistically significant. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows 11.5 (SPSS, Chicago, IL).

### Results

All 20 rats survived the surgical procedures without complications. After the injection of STZ, an increase in blood glucose levels was seen over time, which was accompanied by a reduction in body weight. The mean values of tensile strengths and hydroxyproline levels of the abdominal wounds on the POD 14 of the experiment and the statistical comparisons of the groups are shown in Table 1. The hydroxyproline tissue content of the abdominal fascia in ascorbic acid treatment group was superior to the control group; the difference was statistically significant (P < 0.05). The tensiometric analyses revealed that strength for the midline incision was significantly higher in the study group compared to the control group (P < 0.05). The interobserver (weighted κ 0.70, 95% confidence interval: 0.58–0.71) and intraobserver (weighted κ 0.72; 95% confidence interval: 0.60–0.72) agreements of the scoring system were good.

Acute inflammation score for the study group was higher than that of the control group, and there was a significant difference between the two groups on POD 14.

### Table 1. Comparison of tissue hydroxyproline levels (mg/100 g tissue) and tensile strengths (mmHg) of the abdominal wall between two treatment groups according to POD 10.

<table>
<thead>
<tr>
<th></th>
<th>Groups</th>
<th>Mean ± SD</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile strengths</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>SG</td>
<td>112.6 ± 4.4</td>
<td>0.002</td>
</tr>
<tr>
<td>SG</td>
<td>CG</td>
<td>106.5 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>Hydroxyproline levels</td>
<td></td>
<td></td>
<td>0.034</td>
</tr>
<tr>
<td>CG</td>
<td>SG</td>
<td>9.38 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>SG</td>
<td>CG</td>
<td>9.02 ± 0.6</td>
<td></td>
</tr>
</tbody>
</table>

CG: Control groups
SG: Study groups
*Paired-sample t-test

**Figure 1.** Histological image of SG (A) and CG (B) abdominal wounds 14 days after wounding (Gomori’s trichrome stain) with prominent collagen deposition (A: score 3 out of 3, B: score 2 out of 3).
There was not a significant difference between the two groups in terms of chronic inflammation \((P = 0.511)\). The amount of granulation tissue on POD 14 of the study group was higher than that of the control group and there was a significant difference between the two groups concerning the amount of granulation tissue formation \((P = 0.030)\). Granulation tissue fibroblast maturation on POD 14 in the study group was higher than that of the control group; there was a significant difference between groups \((P = 0.027)\). Collagen deposition (Figure 1) gradually increased on POD 14 of the study group and there was a significant difference between the two groups in terms of collagen deposition \((P = 0.000)\). A significant difference was found between the two groups regarding neovascularization \((P = 0.000)\) [Table 2]. Histological comparisons of SG and CG abdominal wounds at POD 14 after wounding are shown in Figure 2.

**Discussion**

Ascorbic acid has been shown in earlier investigations to have an unparalleled stimulatory effect on collagen-type synthesis. Ascorbic acid is an important modulator of collagen production and acts as a cofactor for the hydroxylation of proline and lysine residues in procollagen.\(^5\)

Wound healing is a complex process that involves inflammation, fibroplasia, neovascularization, collagen deposition, epithelialization, and wound contraction. The normal healing process in healthy individuals takes place at an optimal rate, but it is usually delayed or even impaired completely in patients with diabetes. High blood glucose hinders proliferation of cells and decreases collagen production.\(^3,11\)

Impaired wound healing associated with diabetes mellitus is a significant clinical problem. Two significant parameters of the healing process were evaluated to examine the tissue repair. One was the mechanical strength of the tissues. The other parameter was hydroxyproline level reflecting tissue collagen concentration.\(^12,13\) Hebda et al\(^14\) also evaluated the parameters of wound healing, which include tensile strength of abdominal fascia, tissue hydroxyproline contents, and tissue histologic grading. In the present study, tensile strength and tissue hydroxyproline levels for the laparotomy incision of the study group were higher than the control. Significant increases were detected on POD 14 when measurement of tensile strength and tissue hydroxyproline levels of laparotomy incisions were compared between the study group and the control group.

Wound healing consists of three phases: the inflammatory phase, which consists of inflammatory cell migration to the healing wound; the proliferative phase, which consists of angiogenesis, fibroplasia, epithelialization, and extracellular matrix accumulation; and the final phase, which is maturation.\(^7\) The present study’s histopathologic results indicate that the surgical wound-healing process in laparotomy incisions includes transient acute and chronic inflammation, fibroblast proliferation, neovascularization, and collagen deposition.

Collagen is important in all phases of wound healing and is critical to regaining tissue integrity and strength.\(^15\) Collagen deposition by skin fibroblasts has been shown

**Table 2.** Comparison of histological scoring between the groups according to Abramov’s histologic scoring system.

<table>
<thead>
<tr>
<th>Scores</th>
<th>SG</th>
<th>CG</th>
<th>(P^*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute inflammation</td>
<td>2.6 ± 0.7 (1–3)</td>
<td>1.8 ± 0.9 (1–3)</td>
<td>0.039</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>1.6 ± 0.5 (1–2)</td>
<td>1.4 ± 0.8 (1–3)</td>
<td>0.511</td>
</tr>
<tr>
<td>Amount of granulation tissue</td>
<td>1.8 ± 0.4 (1–2)</td>
<td>1.1 ± 0.8 (1–3)</td>
<td>0.030</td>
</tr>
<tr>
<td>Fibroblast maturation</td>
<td>2.2 ± 0.7 (1–3)</td>
<td>1.5 ± 0.6 (1–3)</td>
<td>0.027</td>
</tr>
<tr>
<td>Collagen deposition</td>
<td>1.8 ± 0.4 (1–3)</td>
<td>1.2 ± 0.2 (1–2)</td>
<td>0.000</td>
</tr>
<tr>
<td>Neovascularization</td>
<td>2.0 ± 0.3 (2–3)</td>
<td>1.4 ± 0.8 (1–3)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

CG: Control group
SG: Study group
*Mann-Whitney U test
to begin within 3–5 days after injury and to continue for several weeks, depending on the size of the wound. Ascorbic acid has been reported to increase collagen deposition in normal animals. In the present study, the levels of hydroxyproline content in the abdominal fascia at the incision site as an indicator of collagen synthesis and wound healing were determined. The results demonstrated a progressive increase in collagen deposition on POD 14 in laparotomy incision.

Myofibroblast proliferation and angiogenesis are important in supporting wound tensile strength. Fibroblasts begin entering the wound site 2–5 days after wounding as the inflammatory phase is ending. Fibroblast numbers peak at 1–2 weeks post-wounding. In the present study, tissue fibroblast maturation was the significant difference between the two groups.

Neovascularization is an important component of the wound-healing process that includes branching and the extension of adjacent blood vessels as well as recruitment of endothelial progenitor cells. Significant increases were detected on POD 14 when neovascularization in laparotomy incisions was compared between the study and control groups.

Conclusion
Administration of ascorbic acid prior to laparotomy expedits wound healing in rats. Ascorbic acid could bestow benefits to tissue healing by significantly enhancing tissue hydroxyproline levels, neovascularization, fibroblast maturation, and collagen deposition. The present finding that ascorbic acid improves wound healing of laparotomy incisions in STZ-induced diabetic rats might be extended to the clinical setting and prove to be an effective promoter of wound healing.

References