# Simvastatin Improves Incisional Wound Healing in a Rat Model: An Experimental Study

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**Abstract:** This study investigated the effect of simvastatin on the healing process of abdominal wall wounds in rats. *Methods.* The study was performed with adult female Wistar-Albino rats. Control group (n = 20) rats were fed standard laboratory diet until 12 hours before surgery. Study group (n = 20) rats received oral simvastatin therapy with an orogastric tube (10 mg/kg once a day) for 7 days until 12 hours before surgery. Each rat was anesthetized, and a 4 cm-long midline laparotomy was performed. Ten animals from each group were killed at postoperative days (PODs) 7 and 14. Breaking strength analysis was measured, and the abdominal incision wounds were examined histologically. *Results.* Hydroxyproline levels and tensile strength of abdominal fascia were significantly higher in the study group on PODs 7 and 14 compared to the control group. The granulation tissue fibroblast maturation scores on POD 7, and both collagen deposition scores and neovascularization scores on PODs 7 and 14, were found to be statistically significantly higher in the simvastatin treatment group compared to the control group, based on the results of the histologic tissue examinations. *Conclusion.* Simvastatin can be used as a supporting therapy in wound healing.

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. Wound healing involves a well-coordinated, highly regulated series of events, including inflammation, tissue formation, revascularization, and tissue remodeling.[1,2] The important role played by nitric oxide (NO) on wound healing has recently been explored.[1,3,4] The upregulation of NO has a positive influence on wound healing at multiple levels, including angiogenesis, inflammation, endothelial and epithelial cell proliferation, matrix deposition, and remodeling.[1,5] Reduced NO production in wounds has been shown to be associated with impaired healing and to coincide with reduced collagen deposition.[5,6]

Statins are known to decrease cholesterol levels, which results in a substantial reduction of cardiovascular mortality in patients with hypercholesterolaemia. Statins reduce cholesterol levels by inhibiting the enzyme 3-hydroxy-

**KEYPOINTS**

- Simvastatin, one of the most common statins, has been demonstrated to be able to augment the secretion of vascular endothelial growth factor (VEGF) in different cell types, and increases NO products in wounds, which can be a power stimulator of angiogenesis.[6]
- The authors hypothesized that simvastatin may aid incisional wound healing in a rat model as a result of its effects on NO upregulation.

3-methylglutaryl-coenzyme A (HMGCoA) reductase, which converts HMG-CoA to mevalonate, the precursor of cholesterol.[7,8] In animal studies, the use of statins on the vascular system, such as the coronary artery, cerebral artery, small mesenteric artery, aorta, and corpus cavernosum, was shown to result in vascular relaxation by upregulating NO synthase.[9,10] Statins have been shown to up-regulate endothelial NO synthase (eNOS) production and subsequent NO bioavailability; this now is believed to be their main mechanism of action.[11] In addition to reducing lipid levels, these agents can improve endothelial function and reduce oxidative stress, which can improve microvascular function.[8-10] Simvastatin, one of the most common statins, has been demonstrated to be able to augment the secretion of vascular endothelial growth factor (VEGF) in different cell types and increase NO products in wounds, which can be a power stimulator of angiogenesis.[6] Matsuno et al[12] showed that simvastatin regulates endothelial regeneration by an over-release of VEGF resulting in a prompt endothelial healing after vascular injury. Recently, Cakmak et al[13] reported that administration of simvastatin therapy had positive effects on the healing of colonic anastomoses. To the authors' knowledge, the effects of simvastatin in an experimental model of incisional wound healing has not yet been investigated. Considering its pharmacological effects, the authors hypothesized that simvastatin may aid incisional wound healing in a rat model as a result of its effects on NO upregulation. The objective of this study was to investigate the effect of simvastatin on the healing process of midline laparotomy via the evaluation of hydroxyproline content, breaking strength, and inflammatory changes in abdominal fascia.

## Methods

**Animals.** The study was performed with 5-month-old, female Wistar-Albino rats (n = 40) weighing between 250 g and 300 g at Ege University Faculty of Medicine (Izmir, Turkey) animal research laboratory. The animals were housed in separate cages in a 22°C–24°C temperature-controlled room with alternating 12-hour light/dark cycles. All experimental manipulations and postoperative care were undertaken in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study also was approved by the Animal Ethics Committee of the Balikesir University Medical School. The 40 rats were randomized into 2 groups of 20. Control group (CG, n = 20) were fed on standard laboratory diet and water *ad libitum* and had free access to water and standard rat feed until 12 hours before surgery. Study group (SG, n = 20) rats were fitted with orogastric tubes for 7 days (10 mg/kg once per day) of simvastatin (Zocor®, Merck & Co, Inc, Istanbul, Turkey) therapy until 12 hours before surgery.

**Operative procedure.** Each rat was anesthetized with intramuscular injection of 60 mg/kg of ketamin hydrochloride (Ketalar, Eczacıbası, 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. Animals were allowed to breathe spontaneously during the surgery. A heating lamp was used to preserve body temperature at approximately 37˚C. The abdominal wall was shaved, the surgical site was scrubbed with povidone iodine, and a 4-cm midline laparotomy was performed. Immediately after, the abdominal fascia and skin were closed in a continuous fashion with running 3-0 silk sutures. All rats were given water and regular diet *ad libitum* on the day of the operation.

Ten animals in each group were sacrificed at postoperative days (POD) 7 and 14 with an overdose of sodium pentobarbital (300 mg/kg, intraperitoneal). The skin sutures were removed and breaking strength of the midline incision was measured (mm Hg) using a tensiometer, as described by Gulcelik et al.[14] Two surgeons, who were blinded to the groups, performed the measurements. Pressure that caused the incision line to separate was defined as the breaking strength. After the breaking 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 2 pieces (2 cm x 1 cm) for 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. All representative fascia sections in each rat were examined histologically under a light microscope by two pathologists in blinded fashion.

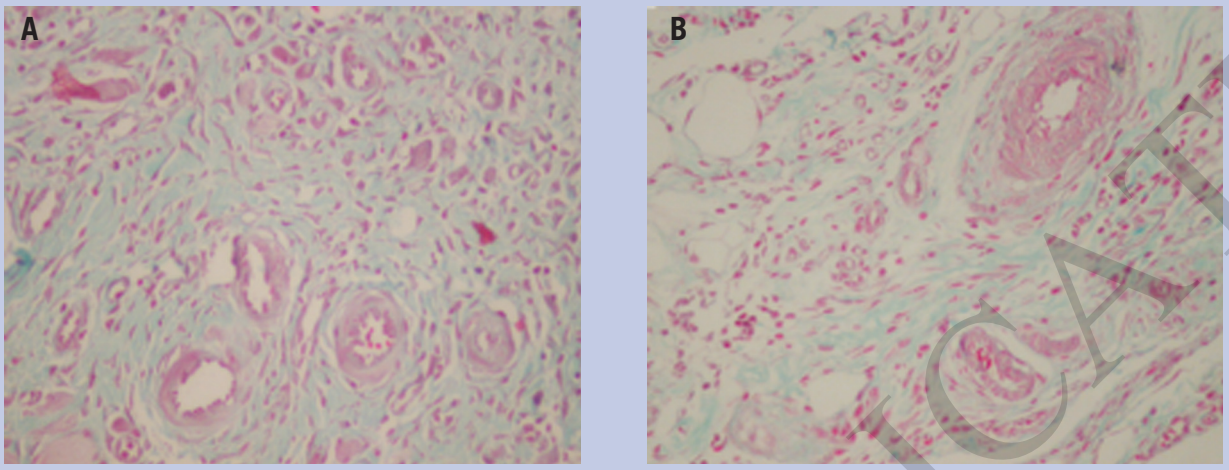


**Figure 1.** Histologic slide of abdominal wounds showing collagen deposition from A) study group and B) control group on POD 14. Study group score = 2 out of 3; Control group Abramov score = 1 out of 3.

Gomori's trichrome stain x100 magnification

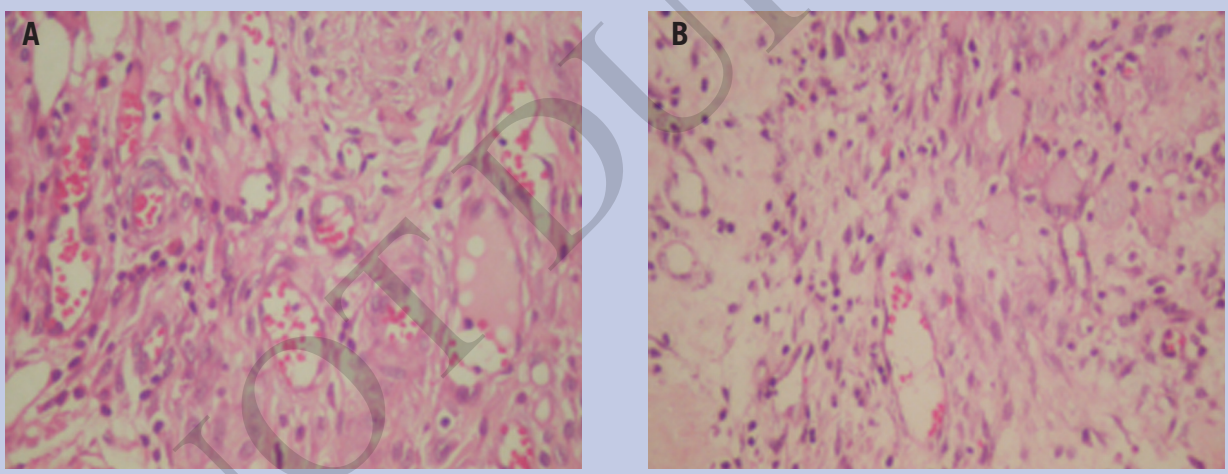


**Figure 2.** Histologic slide of abdominal wounds from A) study group and B) control group on POD 14 with prominent neovascularization. Study group score = 3 out of 3; Control group Abramov score = 2 out of 3.

H&E stain x400 magnification

**KEYPOINTS**

- Study group (SG, n = 20) rats were fitted with orogastric tubes for 7 days (10 mg/kg once per day) of simvastatin (Zocor®, Merck & Co, Inc, Istanbul, Turkey) therapy until 12 hours before surgery.
- After the breaking strength analysis had been completed, the entire incision line, including surrounding intact skin and fascia (1-cm wide) was excised.

**Measuring hydroxyproline.** 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 as mg/100g of tissue.[15]

**Histologic grading.** Biopsy specimens from fascial wounds were obtained as described above on POD 7 and 14. The samples were immediately fixed in formalin,

embedded in paraffin, sectioned, and stained with 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, re-epithelialization, and neovascularization. Acute inflammation was defined as the presence of neutrophils, while chronic inflammation was defined as the presence of plasma and monocytic cells. Abramov's histologic scoring system[16] was used for this study. Two independent pathologists performed the histological examination and applied the scoring system in a blinded fashion.

## Statistical Analysis

The results were expressed as mean ± SD. Interobserver and intraobserver variabilities were calculated using the Cohen's K test. Comparisons between 2 groups were performed using the paired-sample *t*-test, and Mann-Whitney U test. Differences were considered statistically significant when $P < 0.05$. Data were analyzed using SPSS 15.0 statistical software (Chicago, IL) for Windows.

**Table 1.** Comparison of tissue hydroxyproline levels (mg/100-g tissue) and tensile strengths (mm Hg) of the abdominal wall between groups.

| | Groups | Mean ± SD (range) | *P* |
|---|---|---|---|
| POD 7 | | | |
| Hydroxyproline levels | CG | 8.96 ± 0.7 | 0.012 |
| | SG | 9.88 ± 0.6 | |
| Tensile strengths | CG | 92.50 ± 4.9 | 0.001 |
| | SG | 109.12 ± 7.8 | |
| POD 14 | | | |
| Hydroxyproline levels | CG | 9.60 ± 0.5 | 0.004 |
| | SG | 10.82 ± 1.0 | |
| Tensile strengths | CG | 114.68 ± 11.0 | 0.028 |
| | SG | 131.60 ± 16.5 | |

POD: Postoperative day
CG: Control group
SG: Study group

**KEYPOINTS**
- The granulation tissue fibroblast maturation scores on POD 7, including collagen deposition scores and neovascularization scores on POD 7 and 14, were found to be statistically significantly higher in the simvastatin treatment group compared to the control group.
- Results of the present study indicate that simvastatin could be beneficial in tissue healing by significantly enhancing hydroxyproline levels, increasing tensile strength of abdominal fascia, and attenuating inflammatory changes in abdominal fascia.

## Results

All 40 rats survived the surgical procedures with no complications during the study. The mean values of tensile strengths and hydroxyproline levels of the abdominal wounds on the PODs 7 and 14 of the experiment and the statistical comparisons for both groups are shown in Table 1. The hydroxyproline tissue content of the abdominal fascia on PODs 7 and 14 was significantly higher for the SG than the CG ($P = 0.012$ and $P = 0.004$, respectively). The tensiometric analyses revealed that tensile strength for the midline incision was significantly higher on PODs 7 and 14 for the SG than the CG ($P = 0.001$, $P = 0.028$, respectively). The interobserver (weighted K = 0.62, 95% confidence interval: 0.58–0.66) and intraobserver (weighted K = 0.71, 95% confidence interval: 0.60-0.74) agreements regarding the scoring system were satisfactory.

Acute inflammation score, chronic inflammation score on PODs 7 and 14, and the amount of granulation tissue score on POD 14 were higher for the SG than the CG; however, there were no significant differences between the two groups. Granulation tissue fibroblast maturation on PODs 7 and 14 was higher for the SG than the CG, and there was significant difference between the two groups on POD 7 ($P = 0.004$). Collagen deposition (Figure 1) gradually increased on PODs 7 and 14 in the SG, with significant differences noted between groups ($P = 0.035$ and $P = 0.001$, respectively). Neovascularization peaked on PODs 7 and 14 in both groups, and differed significant-

**KEYPOINTS**
- The main histologic outcome measures included the amount of acute and chronic inflammatory infiltrates, the amount and maturation of granulation tissue, collagen deposition, re-epithelialization, and neovascularization.

**Table 2.** Comparison of acute inflammation, chronic inflammation, the amount of granulation tissue, fibroblast maturation, collagen deposition, and neovascularization scores between groups.

| Scores | POD 7 | | | POD 14 | | |
|---|---|---|---|---|---|---|
| | CG | SG | *P* | CG | SG | *P* |
| Acute inflammation | 2.0 ± 0.6 | 2.1 ± 0.7 | 0.754 | 1.3 ± 0.4 | 1.6 ± 0.8 | 0.342 |
| Chronic inflammation | 0.6 ± 0.5 | 0.7 ± 0.4 | 0.660 | 1.6 ± 0.5 | 2.0 ± 0.4 | 0.087 |
| Amount of granulation tissue | 1.4 ± 0.5 | 1.4 ± 0.5 | 1.000 | 1.9 ± 0.5 | 2.1 ± 0.5 | 0.441 |
| Firbroblast maturation | 1.0 ± 0.6 | 1.9± 0.5 | 0.004 | 2.0 ± 0.4 | 2.3 ± 0.4 | 0.177 |
| Collagen deposition | 1.2 ± 0.4 | 1.6± 0.5 | 0.035 | 1.6 ± 0.5 | 2.5 ± 0.5 | 0.001 |
| Neovascularization | 1.3 ± 0.4 | 1.9± 0.7 | 0.045 | 2.1 ± 0.3 | 2.8 ± 0.4 | 0.001 |

POD: Postoperative day
CG: Control group
SG: Study group

ly between groups on PODs 7 and 14 ($P = 0.045$ and $P = 0.001$, respectively; [Figure 2]). Histological comparisons between SG and CG abdominal wounds at 7 and 14 days after wounding are shown in Table 2.

## Discussion

The upregulation of NO has a positive influence on wound healing at multiple levels, including angiogenesis, inflammation, endothelial and epithelial cell proliferation, matrix deposition, and remodeling. Reduced NO production in wounds has been shown to be associated with impaired healing and to coincide with reduced collagen deposition.[17,18] The beneficial effects of NO on wound repair may be attributed to its functional influences on angiogenesis and inflammation.[3,17] Simvastatin increases NO products in wounds, which can be a power stimulator of angiogenesis.Administration of simvastatin therapy has positive effects on wound healing.[8,11,12]

Simvastatin is widely prescribed as a cholesterol-lowering agent. The effect of simvastatin therapy remains central in the long-term management of coronary artery disease and cerebrovascular disease.[6,8] Moreover, simvastatin reduces vascular inflammation and oxidative stress, decreases platelet aggregation, enhances endothelial processes involved in angiogenesis, and promotes angiogenic processes, including endothelial cell proliferation and migration.[6,8,12]

Rego et al[19] reported that application of simvastatin attenuated the inflammatory reaction in healing of infected tissue. Cakmak et al[13] reported the effect of simvastatin on anastomotic bursting pressures and collagen content was significantly higher in a simvastatin treatment group compared to a control group.The authors concluded that simvastatin improved anastomotic healing of the left colon.

The authors hypothesized that simvastatin may lead to an improvement in wound healing.To the authors' knowledge, this is the first study to demonstrate the beneficial effects of simvastatin on histologic characteristics of abdominal wall healing. The parameters of wound healing which include breaking strength of abdominal fascia, tissue hydroxyproline contents, and tissue histologic grading, were evaluated in the present study. PODs 7 and 14 were chosen as the points of measurement for the main incisional study. POD 7 is a relatively early measurement that should detect a delay in the early phases of wound healing. POD 14 is in the middle portion of the curve, and by this point, differences in the rate of primary wound healing should have been evident.[20] The data from this study demonstrate that the administration of simvastatin to rats significantly enhances wound healing by means of increasing mechanical strength and the amount of collagen in abdominal wall tissue.The granulation tissue fibroblast maturation scores on POD 7, including collagen deposition scores and neovascularization scores on PODs 7 and 14, were found to be statistically significantly higher in the simvastatin treatment group compared to the control group. In the present study, the hydroxyproline level in the abdominal fascia at the incision site, considered an indicator of collagen synthesis and wound healing, was determined. The results demonstrated a progressive in-

crease in collagen deposition on POD 7 and 14 in laparotomy incisions. The Abramov's histologic scoring system[16] was used in the present study. Correlating this scoring system with objective measures, such as hydroxyproline content for collagen deposition, polarized light microscopy for assessment of granulation tissue maturation, and immunohistochemistry for characterization of inflammatory cells, were all beyond the scope of this study, and should be considered in future research.

## Conclusion

The findings show that simvastatin can be used as a supporting factor in wound healing. Results of the present study indicate that simvastatin could be beneficial in tissue healing by significantly enhancing hydroxyproline levels, increasing tensile strength of abdominal fascia, and attenuating inflammatory changes in abdominal fascia. Histopathological analysis revealed that simvastatin administration leads to a better wound healing in terms of fibroblast maturation on POD 7 and both collagen deposition and neovascularization on PODs 7 and 14. The present study is one of the first to demonstrate that simvastatin significantly improves healing of incisional wounds in an experimental rat model. Further clinical studies are needed to clarify the usefulness of simvastatin for healing wounds.

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