The Effects of Single-Dose Dexamethasone on Wound Healing in Rats

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Dexamethasone effectively decreases the incidence of nausea and vomiting among pediatric and adult patients. In this study, we evaluated the effects of single-dose dexamethasone on wound healing in a prospective, randomized, experimental animal model. Anesthesia was induced with thiopental 100 mg/kg intraperitoneally. Dexamethasone 1 mg/kg was administered intraperitoneally in a dexamethasone group, and physiological saline was administered in a control group. Collagenization, epithelization, and fibroblast content were significantly less in the dexamethasone group compared with the control group (*P* values of 0.002, 0.041, and 0.023, respectively). The vascularity and the degree of inflammatory cells were more intense in the dexamethasone group compared with the control group (*P* values of 0.023 and 0.002, respectively). The white blood cell count was similar in the control (7.84 ± 2.09) and dexamethasone (6.98 ± 2.12) groups. The mean hydroxyproline level was 0.72 ± 0.13 mg/g in the dexamethasone and 1.03 ± 0.19 mg/g in the control group. Hydroxyproline levels were significantly less in the dexamethasone group ($P = 0.001$). We conclude that dexamethasone at 1 mg/kg may have negative effects on wound healing.

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Postoperative nausea and vomiting remains a common distressing problem after anesthesia and surgery, despite the use of currently available antiemetics. Several studies have implied that dexamethasone effectively decreased the incidence of nausea and vomiting among pediatric and adult patients (1,2). Dexamethasone 1 mg/kg (maximum, 25 mg) is an effective prophylactic antiemetic for postoperative nausea and vomiting in children undergoing adenotonsillectomy and strabismus repair (3,4). Dexamethasone has antiinflammatory effects and has been used during operations for decreasing edema formation and swelling and for preventing ischemia-reperfusion injury (5–7).

Corticosteroids markedly affect most aspects of wound healing. When corticosteroids are administered early after injury, high corticosteroid levels delay the appearance of inflammatory cells and fibroblasts, the deposition of ground substance and collagen, regenerating capillaries, contraction, and epithelial migration (8–10). The aim of this study was to evaluate the effects of single-dose dexamethasone 1 mg/kg on wound healing in a prospective, randomized, experimental animal model.

Methods

The study was approved by the Inonu University School of Medicine Animal Care and Use Committee. Experiments were conducted on male Wistar albino rats weighing 250–300 g. In a temperature-controlled and ventilated room with a 12-h light-dark cycle, animals were housed for at least 10 days before experiments and were given unlimited access to food and water. On the experiment day, rats were randomly assigned to two groups of eight animals each. Anesthesia was induced with thiopental 100 mg/kg intraperitoneally (IP). Dexamethasone 1 mg/kg IP was administered in the dexamethasone group, and physiological saline was administered in the control group. The hair was closely shaved with an electrical razor, and the surgical field was disinfected with povidone-iodine and draped with sterile towels. All surgical procedures were performed under aseptic conditions by the same surgeon. A dorsal midline incision, measuring approximately 4 cm, was made through the

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skin of each animal until the muscular fascia was exposed. Then the dorsal wound margins were apposed with a nonabsorbable interrupted suture. No postoperative antibiotics were given. During the experiments, the animals were provided *ad libitum* with bottled tap water and the institute's stock diet for rats.

On the 14th day, the dorsal wounded area was cut into a 5 × 1 cm strip under 100 mg/kg IP thiopental anesthesia. For histopathologic examinations, 1-cm^2 pieces of wound were obtained from the caudal part. The biopsies were placed in 10% formaldehyde, embedded in paraffin, sectioned perpendicular to the wound, and stained with hematoxylin-eosin for later analysis. Masson's trichrome dye was used to determine collagenization. The remaining skin was used for hydroxyproline level determination. All rats were killed after a 3-mL blood sample was obtained for white blood cell count.

Histopathologic samples were examined by using light microscopy (20×) and scored by using a modified Ehrlich-Hunt numerical scale (11). Fibroblast content, collagen deposition, vascularity, and inflammatory cell infiltration were graded as 0 for absence, 1 for occasional presence and light scattering, 2 for abundance, and 3 for confluence of cells and fibers. Epithelial regeneration was scored as 0 for no epithelium, 1 for single-layer epithelium with partial closure, and 2 for multilayer epithelium with complete closure (12). A light microscope equipped with polarized light optics was used to determine the birefringence intensity of the collagen fibers. The same pathologist, who was blinded to the sections belonging to groups, performed histopathologic examinations.

The tissue samples taken for hydroxyproline determination were washed with physiological saline and dried for 72 h in an etuve adjusted to 100°C. Hydroxyproline levels were determined spectrophotometrically with Woessner's method (13) after samples were weighed and hydrolyzed in concentrated hydrochloric acid (12 N HCl) at 130°C for 3 h. After each sample was adjusted to a final volume of 1 mL, samples were centrifuged at 3000*g* for 15 min to obtain supernatant. A second centrifugation at 2500*g* for 10 min was performed after isopropanol addition to an equal volume of supernatant. Serial dilutions of commercial pure hydroxyproline (Sigma) were used as standard. Hydroxyproline concentrations of the samples were calculated by using the absorbance-concentration curve of standard hydroxyproline solutions.

Parametric data were analyzed by one-way analysis of variance. Differences between groups were analyzed with independent-samples Student's *t*-tests. Mann-Whitney *U*-tests were used for data regarding collagenization and epithelization because variance homogeneity was not obtained. Differences were considered statistically significant at $P < 0.05$.

Table 1. The Median Histopathologic Scores Determined in the Control and Dexamethasone Groups

Variable	Control	Dexamethasone
Epithelization	2 ± 0.35	1 ± 0.52
Fibroblast content	2 ± 0.52	2 ± 0.45
Inflammatory cells	1 ± 0.35	2 ± 0.52
Vascularity	2 ± 0.52	2 ± 0.45
Collagenization	2 ± 0.53	1.5 ± 0.45

Data are expressed as median ± SD.

Results

During the observation period, no animals died in the control or dexamethasone groups. Collagenization, epithelization, and fibroblast content were significantly smaller in the dexamethasone group compared with the control group (*P* values of 0.002, 0.041, and 0.023 respectively). The vascularity and the degree of inflammatory cells were higher in the dexamethasone group compared with the control group (*P* values of 0.023 and 0.002, respectively). The median histopathologic scores determined in the control and dexamethasone groups are shown in Table 1. A photomicrograph of an animal from the control group and an animal from the dexamethasone group are shown in Figure 1.

The white blood cell count was similar in the control (7.84 ± 2.09) and dexamethasone (6.98 ± 2.12) groups. No significant difference was determined in the mean percentage of neutrophils (24.13% ± 10.44% versus 30.88% ± 6.40%), lymphocytes (69.33% ± 10.87% versus 63.50% ± 4.78%), and monocytes (3.90% ± 3.43% versus 2.76% ± 1.51%) in the control and dexamethasone groups, respectively.

Hydroxyproline levels were 0.72 ± 0.13 mg/g in the dexamethasone group and 1.03 ± 0.19 mg/g in the control group. Hydroxyproline levels were significantly less in the dexamethasone group ($P = 0.001$). The levels determined in the control and dexamethasone groups are shown in Figure 2.

Discussion

We found that a single dose of dexamethasone given to prevent postoperative nausea and vomiting has a deleterious effect on wound healing. The essential phase of wound healing is the inflammatory phase, characterized by increased vascular permeability, chemotaxis of the cells from circulation into the wound milieu, local release of cytokines and growth factors, and activation of migration cells (10). In previous studies, corticosteroids reduced inflammation, which affects cell migration, proliferation, and angiogenesis (14). Corticosteroids inhibit the inflammatory phase, which causes delayed wound healing. Corticosteroids

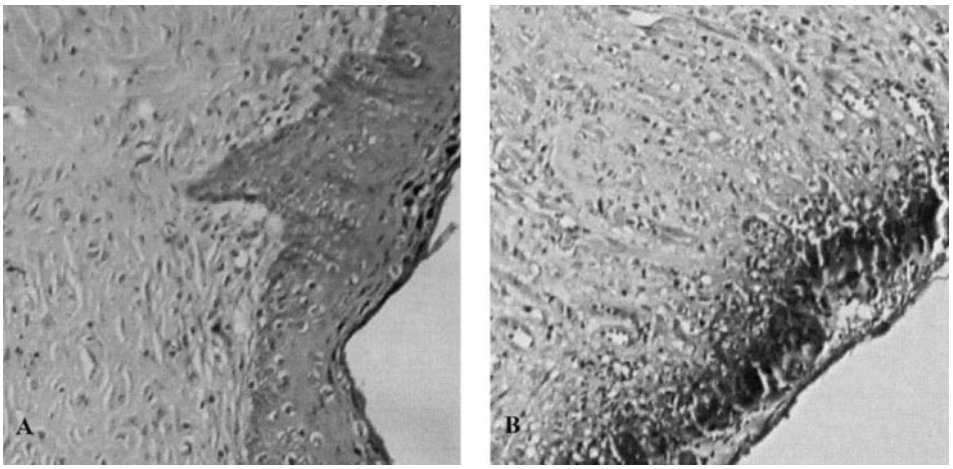

Figure 1. Photomicrographs of the study groups. A, Control group. B, Dexamethasone group. Collagenization, epithelization, and fibroblast content were less in the dexamethasone group. The vascularity and the degree of inflammatory cells were more intense in the dexamethasone group.

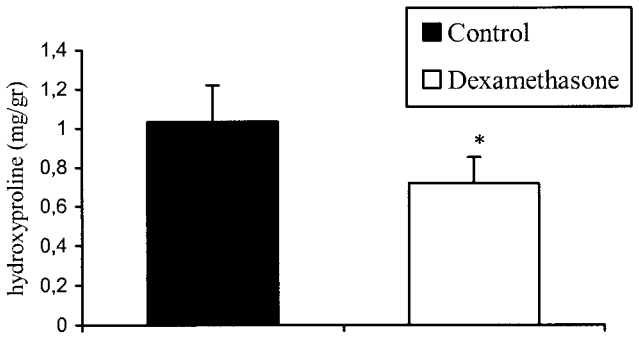

Figure 2. Mean hydroxyproline levels determined in the control and dexamethasone groups. * $P < 0.01$.

also inhibit collagen synthesis in wounded tissues and, therefore, have been used for treatment of corrosive esophageal burn to prevent stricture formation (15–17). Corticosteroids also decrease collagen synthesis both in unwounded connective tissues and in fibroblast cell culture. The decrease of Type I collagen synthesis caused by steroids has been attributed to a decrease of the steady-state level of total cellular Type I procollagen messenger RNAs. Glucocorticoids regulate α_2-Type I procollagen promoter activity (18).

The synthesis of the 19 known collagens occurs within the cell, as it does for other proteins. The collagen molecule is characterized by the repeating sequence Gly-X-Y, with X often being proline and Y often being hydroxyproline (19). Hydroxyproline is the end product of collagen breakdown. For this reason, tissue hydroxyproline level is an indirect and objective variable of tissue collagen production. In many experimental studies, hydroxyproline has been used to assess tissue collagen production (20–22).

Because of the importance of timing for prophylactic antiemetic administration, we administered the drugs at the beginning of the procedure. It has been confirmed that dexamethasone is more effective for preventing postoperative nausea and vomiting when administered at the induction of anesthesia than when it is given at the end of surgery (23).

The wound-healing process has been conveniently divided into three phases—inflammatory, proliferative, and remodeling. However, the process is continuous, and phases overlap. Therefore, the conceptual distinction between phases serves only as an outline to discuss events that occur during wound repair. The presence of more mature capillary vessels in the vicinity of a wound allows for better nutrition, and this phenomenon, combined with a large amount of collagen fiber, is directly related to a more adequate wound-healing process (24). Angiogenesis is a dynamic process during wound healing, as the fibrin clot is replaced by blood vessel-rich granulation tissue and is subsequently replaced by a collagenous scar with much less mature vessels (25,26). In our study, there were significantly more inflammatory cells and vascularity in the dexamethasone group. The presence of significant inflammatory cells and vascularity in the dexamethasone group compared with the control group might be related to delayed inflammatory and proliferation phases. Increased collagenization and epithelization with fewer inflammatory cells and less vascularity provided evidence of repletion of granulation tissue to collagenous scar in the control group because rat wound healing was rapid (20).

Although dexamethasone is a cost-effective antiemetic and has been widely used, the delayed wound-healing process suggests that dexamethasone should be avoided in patients with poorly healing wounds or leg ulcers or when fast healing is essential. In such patients, retinoic acid administration to the treatment protocol may improve the healing process. In a study by Wicke et al. (9), retinoic acid significantly increased the hydroxyproline content toward normal levels in approximately 80% of controls at Day 17. Further studies should be performed after a single-dose dexamethasone administration to determine the effects of

retinoic acid on wound healing. It must be remembered that steroids and retinoic acid have regulatory effects for the synthesis of collagen, even in the early phase of wound healing (10).

In conclusion, this study has shown that dexamethasone at 1 mg/kg doses may have negative effects on wound healing. To substantiate the dose-related effects, further experiments with dexamethasone at different doses will be required.

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