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The effects of locally applied procaine on wound healing

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ABSTRACT

Objective: The goals of this study were: (1) To determine the efficacy of 2% procaine (the most commonly used concentration) in wound healing; and (2) To determine the proper open wound injection site.

Materials and Methods: Thirty adult male Sprague-Dawley rats weighing between 250 and 350 g were used. Two full thickness defects were made on two sides of the midline 1 cm away from midline. The skin wound areas were approximately 1.5 cm × 1.5 cm. The animals were randomly divided into three groups: Group 1 (control group, n = 8), Group 2 (injection directly into the base of wound, n = 8), and Group 3 (injection into healthy skin around the peripheral margins of the wound, n = 8). Mechanical analyses of wound tensile strength of were evaluated in all groups.

Results: Wound closure was first seen in Group 3 on day 14. Mean wound healing times were 18.25 days, 16.25 days, and 15.62 days, and mean tensile strength was 777.13 cN, 988.25 cN, and 1068.25 cN in the Groups 1, 2, and 3 respectively.

Conclusions: Procaine did not cause any necrosis around the wound, did not retard wound healing, did not cause circulation deficiency, and did not reduce the breaking strength of the wound. Therefore, it can be safely used to reduce pain around the wound and to accelerate the healing process of slow-to-heal wounds.

Key words: *Procaine, slow-to-heal wounds, tensile strength*

Introduction

Procaine hydrochloride (also known as novocaine) was first synthesized by the German chemist, Alfred Einhorn, in 1905 and was used as a local anaesthetic. In the 1950s, doctors discovered its "anti-aging" effects as well. Preparations that contain procaine as a component claim to have the following beneficial effects: Neuron regeneration, cell membrane modulation, protection against cerebral anoxia, antioxidant activity, increasing resistance to infections and toxins, antidepressant activity (as a reversible inhibitor of monoaminoxidase), increasing serum high density lipoprotein -cholesterol concentrations, and decreasing triglyceride levels [1]. Mesotherapy cocktails, which are used as a noninvasive alternative to lipoplasty, a treatment for cellulite, and for body sculpting and skin rejuvenation, include procaine due to its vasodilatator and analgesic effects [2]. In addition, procaine reduces the symptoms of carpal tunel syndrome (CTS) and leads to improved electrophysiologic findings following steroid injection [3].

Some researchers have stated that local anesthetics retard wound healing, whereas others claim that anesthetics have a positive effect on the healing process [4,5]. In a literature review, we found only one article about the effects of procaine on wound healing [6], where the authors used a wound healing primer and found that procaine retarded wound healing in rats that were administered 2% procaine with adrenaline. These effects have been attributed to a reduction in the synthesis of mucopolysaccharides, and most lkely collagen as well [6].

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Currently, several reconstructive surgeons use local anesthetics in the management of pain. In addition, studies have found that local anesthetics should be avoided in slow-to-heal wounds, leg ulcers, or anywhere that fast healing is essential [4]. In the present study, a full thickness excisional wound model was used to determine the efficacy of 2% procaine (the most commonly used concentration) in open wounds and to determine the proper open wound injection site, whether it should be injected into healthy skin around the peripheral margins of the wound or directly injected into the base of the wound.

Materials and Methods

Thirty adult male Sprague-Dawley rats weighing between 250 and 350 g were used in this study. The rats were housed in separate cages under standard laboratory conditions (12 h of light/12 h of darkness, 17-25°C, 30% humidity, ad libitum access to chlorinated tap water and rat chow) throughout the experiment. The ethical board of the Şişli Etfal Training and Research Hospital gave consent for the study.

On the day of surgery, animals were anesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg). The backs of the rats were shaved with electric clippers. All surgical interventions were performed under sterile conditions, and povidone iodine was used as an antiseptic. Two full thickness defects were made on two sides of the midline 1 cm away from midline. Skin wound areas were approximately 1.5 cm × 1.5 cm. The excised tissues included the epidermis, dermis, and panniculus carnosus. A biopsy specimen was taken from one defect and used for pathologic examination; the other defect was used for tensile strength testing. All wounds were immediately dressed with sterile sponges to induce post-operative hemostasis, and sterile tegaderm transparent dressing was used as well (3M Health Care, St. Paul, Minnesota, USA). Antibiotics were not given post-operatively [Figure 1]. After removing the dressing on the 1st day, all wounds were left to heal by secondary intention until 30 days post-surgery. The animals were randomly divided into three groups: Group 1 (control group, n = 8) did not receive a pharmacologic agent, and was simply followed-up; Group 2 (direct injection into the base of the wound; n = 8) and Group 3 (injection into healthy skin around the peripheral margins of the wound; n = 8) received 2% procaine

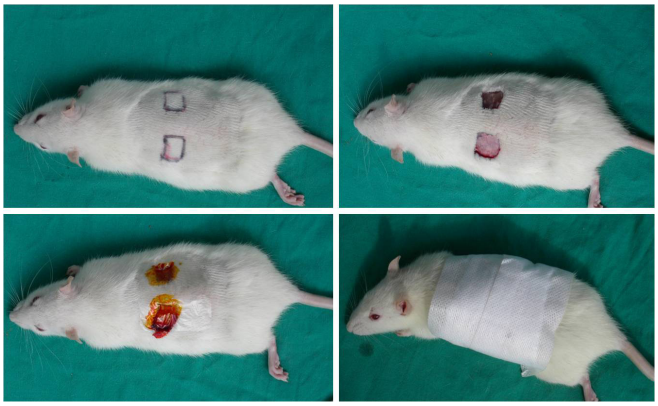


Figure 1. The backs of the rats were shaved. Two full thickness defects were made on two sides of the midline 1 cm away from midline. Skin wounds areas were 1.5 cm × 1.5 cm. The excised tissues included the epidermis, dermis, and panniculus carnosus.

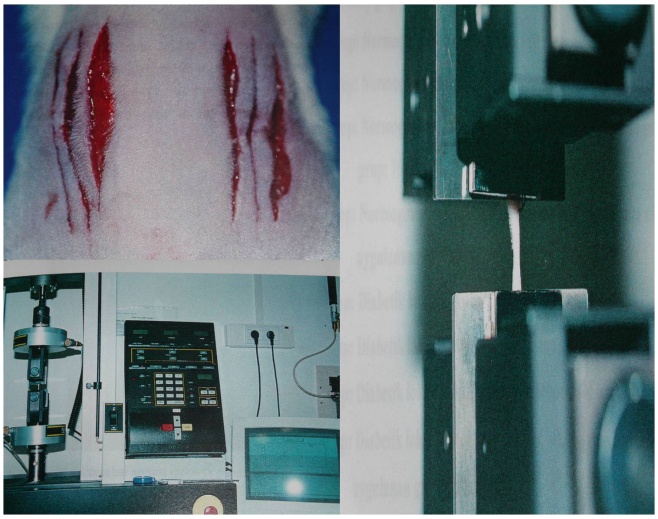


Figure 2. On the 30th post-operative day, 0.5 cm × 3 cm strips of healed wounds were oriented perpendicular to the long axis of the body. These strips were placed on a tensiometer for mechanical strain testing.

at a dose of 50 mg/kg per day for 14 days.

Wound edges were evaluated by visual inspection, and time for complete epithelization was recorded on the 0, 7th, 14th, and 21st post-operative days. On the 21st post-operative day, biopsy specimens were taken from the healing wound sites and surrounding healthy skin for microscopic evaluation. On each post-operative day, eight specimens were taken for all groups. All specimens were stained with hematoxylin and eosin, and inspected under light microscopy by the same pathologist who was blinded to the data. Neovascularization, inflammation, re-epithelization, fibroblast migration, and collagen deposit were evaluated as parameters. On the 30th post-operative day, 0.5 cm × 3 cm strips of healed wounds oriented perpendicular to the long axis of the body were uniformly excised, including the scar tissue. There were eight strips in all groups, which were placed

on a tensiometer (Instron Corporation Series, IX Automated Materials Testing System, Norwood, MA, USA.) for mechanical strain testing. Values were recorded in cN. Specimens were held in non-slip jaws and stretched apart at a constant speed of 6 cm/min until they ruptured [Figure 2]. The tensile strength of the strips was recorded in grams.

Data were assessed by Kruskal–Wallis and Mann–Whitney U-tests. $P < 0.05$ were considered statistically significant. Statistical analysis was performed with SPSS software version 11.0 (Chicago, IL, USA).

Results

Wound healing days were recorded in each group macroscopically. The first wound closure was seen in Group 3 on day 14. The mean wound healing times were 18.25 days, 16.25 days, and 15.62 days in Groups 1, 2, and 3, respectively [Table 1]. The rate of healing in each group was compared by the Kruskal–Wallis test. The differences between groups were highly statistically significant ($P < 0.001$). When all groups were compared with each other by the Mann–Whitney U-test, the difference between the two experimental groups and the control group was statistically significant ($P < 0.005$), although the difference between the two experimental groups was non-significant ($P > 0.05$) [Graph 1].

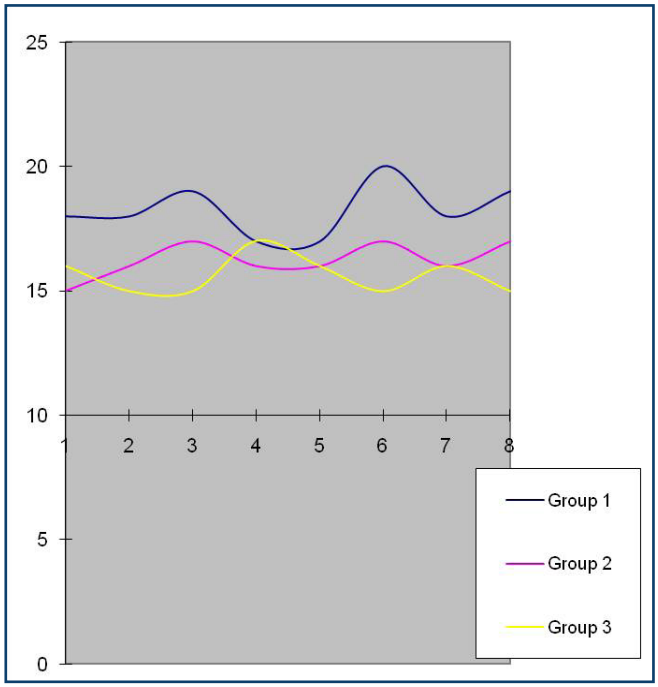
There were no significant differences in neovascularization, inflammation, and fibroblast migration at day 21 in all groups. Collagen accumulation was better in the experimental groups than the control group. Epithelization was completed in all groups [Figure 3]. In the control group, complete re-epithelization was not seen.

Mechanical analyses of wound tensile strength were evaluated in all groups, and mean tensile strength val-

Table 1. Number of wound healing days in each group.

Number	Group 1	Group 2	Group 3
1	18	15	16
2	18	16	15
3	19	17	15
4	17	16	17
5	17	16	16
6	20	17	15
7	18	16	16
8	19	17	15
Mean±SD	18.25±1.03	16.25±0.7	15.62±0.7

SD: Standard deviation



Graph 1. The differences between groups were highly statistically significant ($P < 0.001$), as determined by the Kruskal–Wallis test. When all groups were compared with each other using the Mann–Whitney U-test, the difference between the two experimental groups and the control group was statistically significant ($P < 0.005$); however, the difference between the two experimental groups was non-significant ($P > 0.05$).

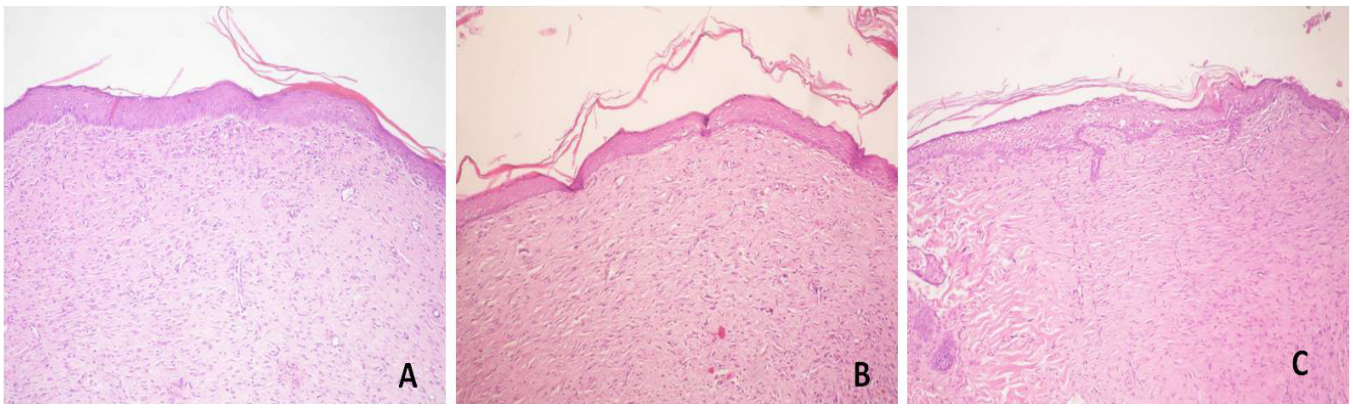
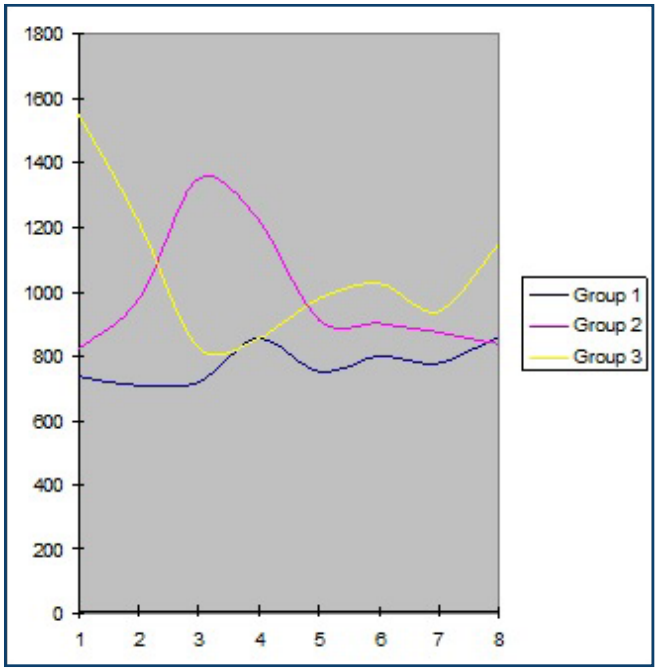


Figure 3. There were no significant differences in neovascularization, inflammation, and fibroblast migration on day 21 in all groups. Collagen accumulation was better in the experimental groups than the control group.

Table 2. Breaking strength of the each group.

Number	Group 1	Group 2	Group 3
1	738	825.0000	1550.000
2	710	982.0000	1214.000
3	720	1350.0000	830.000
4	856	1225.0000	856.000
5	752	910.0000	980.000
6	802	902.0000	1028.000
7	780	873.0000	940.000
8	859	839.0000	1148.000
Mean	777.125	988.2500	1068.250



Graph 2. There were no statistically significant differences between groups and subgroups.

ues were 777.13 cN, 988.25 cN, and 1068.25 cN in the Groups 1, 2, and 3, respectively. Tensile strength in all groups is shown in Table 2. There were no statistically significant differences between groups, as determined by the Kruskal-Wallis test ($P > 0.001$), and no statistically significant differences between subgroups, as determined by the Mann–Whitney U-test ($P > 0.05$) [Graph 2].

Discussion

Recently, surgeons have began playing an active role in modulating the healing process with the pharmological treatment of open wounds. Procaine has widespread usage in clinical practice and is also a component of mesotherapy cocktails due to its vasodilatator and analgesic effects in anti-aging agents [1-3]. However, in the United States, some agents that containe procaine and are used for dementia were banned by the Food and Drug Administration [1]. Procaine also relieves CTS because it has potent local anesthetic properties [3]. Despite this knowledge about procaine, the literature lacks information about its effects when injected into a wound or into healthy tissues around the wound.

A full thickness excisional wound model was used to detect the effects of procaine on wound healing. Open wounds heal by the same basic processes of inflammation, proliferation, and remodelling as do closed wounds. The major difference is that each sequence is much longer in open wounds [7]. It has been stated that injection of sterile water into the wound region delays wound healing, presumably because the volume of liquid injected into the skin and subcutaneous tissues causes tissue damage [7]. Thus, sterile water was not used in the control group. It has been shown that procaine retards wound healing by reducing mucopolysaccharide, and consequently, collagen synthesis [8]. Many hospitals are now using local anesthetics to relieve post-operative pain and prolong analgesia, although some studies about local anesthetics suggest that these agents should be avoided in patients with slow-to-heal wounds, leg ulcers, or when fast healing is essential [9,10]. Studies have shown that 1% and 2% concentrations of procaine delayed wound healing, and the use of 1:1,00,000 epinephrine was a factor that exacerbated this effect by allowing procaine to remain in the region longer. Thus, epinephrine injected alone in the region had no negative effect on the healing of wounds [5]. In the present study, procaine that was injected into the base of the wound and around the wound did not retard healing times and rates.

Some reports have stated that local anesthetics preserve endothelial barrier function during acute inflammation. The effects of local anesthetic agents on cytokine release and activity are dose-dependent [11]; however, it is known that local anesthetics inhibit both cytokine release and activity. Nevertheless, when one considers the diverse array of cytokines and cytokine effects involved in the inflammatory response and their complex interactions, it is clear that our knowledge of the effect of local anesthetic agents remains limited. Ester and amide local anesthetics decrease leukocyte migration [12] and are associated with decreased end organ damage. In ad-

dition, some studies have reported that local anesthetic agents decrease leukocyte migration [13] and inhibit generation of hydrogen peroxide. Local anesthetics may function by altering the distribution (rather than the magnitude of expression) of adhesion molecules. The limitation of our study was that we did not investigate leukocyte migration by microscopy, and did not perfom immunohistochemistry for adhesion molecular markers. However, both procaine treatment groups healed uneventfully, and had a shorter healing time than the control group. Also, collagen accumulations in experimental groups were more prominent.

The infiltration of local anesthetics at high concentrations (2%) decreases the breaking strength of skin wounds in rats, but at a concentration of 0.5%, the differences are not significant [6]. In our study, 2% procaine did not delay wound healing and did not lead to significant differences compared to the control group. In addition, there were no statistically significant differences between experimental groups.

In the full thickness excisional model, healing rates are often monitored based on the extent of re-epithelization, histological organization of connective tissue, angiogenesis, and biochemical content of collagen or proteoglycans [14]. In this study, we found that procaine did not cause any necrosis around the wound, did not retard wound healing, did not cause circulation deficiency, and did not reduce the breaking strength of the wound. Thus, this agent may be recommended for the healing of both open wounds and surgical wounds due to its effect on collagen accumulation. In addition, procaine can be used safely to reduce pain around the wound and to accelerate the healing of slow-to-heal wounds.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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