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Original Article

The Effects of Topical Insulin Application on Wound Healing

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ABSTRACT

The process of wound healing is a dynamic event during which the stages of fibroplasia, angiogenesis, and re-epithelization perfectly take place.The aim of this study is to compare the effects of wound irrigation by normal saline and topical insulin application, which we frequently use in clinical practice, on wound healing. The study covers a total of 20 male rats -10 for the insulin group and 10 for the control group. The first group received topical insulin application while the second group had irrigation by normal saline. The macroscopic outlook, collagen production, and wound contraction rates in the animals wounds were checked at the end of day 20. The rate of wound closing was found to be higher in the topical insulin group than the NS group at all times. Further, the period of complete wound closing was shorter than the insulin group. Histopathological analysis revealed that the ulceration and inflammation were localized in the subepithelial field in the skin cross-sections of the insulin group and that there was a significant increase in collagen bundles. Thus, we think that insulin can be an alternative to normal saline application specifically in chronic wounds related to diabetes and post-op wound care.

Key words: *Topical insulin, wound healing, chronic wound*

Topikal İnsülin Uygulamasının Yara İyileşmesi Üzerine Etkileri

ÖZET

Yara iyileşmesi süreci fibroplazi,anjiogenesis ve re-epitelizasyon aşamalarının kusursuz şekilde oluştuğu dinamik bir olaydır.İnsülin bu basamakların tümünde rol alarak yara iyileşmesini hızlandırır.Bu çalışmamızda klinik pratikte sık kullandığımız serum fizyolojik ile yara irrigasyonu ve topikal insülin uygulamasının yara iyileşme üzerine etkilerinin karşılaştırılması amaçlanmıştır. Çalışmaya insülin grubu 10 adet ve kontrol grubu 10 adet rattan oluşan toplam 20 adet erkek rat dahil edildi.Birinci gruba topikal insülin uygulaması ve ikinci gruba serum fizyolojik ile irrigasyon yapıldı.10.günün sonunda hayvaların yaralarındaki makroskopik görünüm,kollajen üretimi ve yara kontraksiyon oranlarına bakıldı. Topikal insülin uygulanan grupta yara kapanma oranı tüm günlerde SF uygulanan gruptan daha yüksek bulundu.Ayrıca tam yara kapanma süresi insülin grubundan daha kısaydı..Histopatolojik incelemede insülin grubunda; cilde ait kesitlerde, ülserasyon ve enflamasyonun subepitelyal alanda lokalize olduğu, kollagen demetlerinde ciddi bir artış olduğu görüldü.SF grubunda ise inflamatuar infiltrasyon bölgesinin altında granülasyon dokusu ve ülser sınırlarında belirgin olmak üzere fibröz kollajenöz skar dokusu izlendi. İnsülin uygulanan ratlarda kontrollere göre ülser derinliğinin ve inflamasyonun azalmış olması ve skar dokusunun yoğun olarak gözlenmesi insülinin yara iyileşmesini hızlandırdığını göstermektedir.Bu nedenle özellikle diyabete bağlı oluşan kronik yaralarda ve postoperatif yara bakımında insulin serum fizyolojik uygulamasına alternatif olabileceği kanısındayız.

Anahtar Kelimeler: *Topikal insülin,yara iyileşmesi,kronik yara*

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INTRODUCTION

Wounds can be the results of trauma or surgical incisions. Further, pressure ulcers are a type of skin ulcer and can be regarded as wound. An inflammatory response takes place following injury. The most important part in inflammatory response is played by macrophages. Macrophages reproduce in the wound bed and secrete mediators against the pathogens in the wound and debride the wound. The extracellular matrix which is needed for the epithelization is produced during the fibroplasia stage and through the proliferation of myofibroblasts wound contraction is enabled. This stage is the most important stage of wound healing (1,2). Epidermis, too, plays an important part in wound healing by enabling a barrier against infections and through its role in homeostasis. The keratinocytes around the wound are differentiated during the re-epithelization stage (3,4). The differentiation takes places simultaneously in the epidermis and dermis, not in levels, during re-epithelization.

Insulin enhances the extracellular matrix and collagen synthesis by increasing the proliferation of myofibroblasts during the fibroplasia stage by elevating the macrophage activation through some ways in the inflammatory phase. It accelerates all phases of wound healing by increasing the keratinocyte differentiation, migration, and proliferation in the re-epithelial stage (5,6). Insulin treatment in wound healing has been tried systematically in the treatment of leg fractures of horses, in the treatment of skin ulcer in diabetic and non-diabetic rats, in the wound treatment of diabetic individuals, and in burn treatment (7-10). Moreover, insulin's contribution to fibroblast growth and granulation tissue formation has been demonstrated in in vivo experimental culture studies (11). There is proof that systemic insulin application has a positive effect on diabetic wound healing (12,13). Some studies, however, present contradictory results regarding the effect of topical insulin application on wound healing (14,15). In this study we evaluated the effects of topical insulin application in order to clarify the effects of insulin applied topically in rats with experimental wounds.

MATERIALS AND METHODS

An ethics board approval (no 2012-68) was obtained from Konya Necmettin Erbakan University's Experimental Studies Center before the study. A total of 20 healthy male Winstar albino rats weighing 210-340 gr were covered by the study. Each rat was placed in a separate plastic cage and classic ad libitum feed and enough water was secured in the cages during the study. 100 mg/kg ketamine HCI (Ketalar vial, Parke-Davis, Morris Plains) and Xylazine HCI (Rompon vial, Bayer) in 25 mg/kg concentration were administered intraperitoneally (IP) to all the rats. The rats' backs were shaved by an epilator. Following the cleansing of the incision area with 10% povidone iodine, full layer dermis and epidermis incision of 10 x 4 mm was obtained by the application of 90oC electric cautery for 20 seconds. The rats with formed wounds were divided into two groups. 0.3 IU units of regular insulin diluted with 20 µL sterile water was applied to the lesions of 10 rats in a single dose covering the wound area at 08:00 every day, while 40 µL normal saline was applied to the lesions of 10 rats making up the control group for 20 days. The rats wound measurements and full epithelization conditions were evaluated before medicine application every other day. All the rats were anesthetized by IP 100 mg/kg ketamine HCI and Xylazine HCI in 25 mg/kg concentration at the end of day 20. The diameters and depths of the rats' lesions were measured. Wound closing rates were calculated according to the following formula (8):

$$\text{Closing \%} = \frac{\text{Area at day 0 (length X depth)–open area at day n}}{\text{Area at day 0}} \times 100$$

Further, full epithelization was visually observed on the wound. No open areas were taken to be the full epithelization criteria. Tissue samples were taken from all the lesions for pathological study. The samples were fixed in 10% formaldehyde. Then routine paraffin burial procedure was carried out. Following dehydratation, the blocks were cleansed through a series of ethyl alcohol and toluene procedures. 5-6 µm thick sections were taken out of the blocks buried in paraffin and were stained in hematoxylin eosin for histological study and in Masson's trichrome stain for the evaluation of collagen distribution.

RESULTS

No animal death was observed during the course of the study. The rate of wound closing was found to be higher in the topical insulin group than the NS group on all days. Moreover, complete wound closing period was shorter for an average of 4 days (Table 1). Complete epithelization took place in 1 out of 10 rats on day 7 (10%), in 9 rats on day 11 (90%), and in all rats on day 13 in the insulin group.

Table 1. Wound closing rates of the insulin and NS groups. Median rate (minimum rate-maximum rate)

	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17
Insulin	28.7 (10-40.6)	54.6 (41.2-75.6)	79.8 (72.05-92.26)	94.6 (89.4-97.2)	99.5 (99.2-100)	100 (100-100)	100 (100-100)	100 (100-100)
NS	27.8 (8.82-8.6)	42.3 (25.3-56.1)	55.4 (32.5-64.3)	78.1 (62.7-88.2)	88.1 (76.5-93.7)	95.8 (92.5-98.4)	98.2 (97.6-99.6)	100 (100-100)

While no cases of complete epithelization was seen in none of the rats in the NS group on day 7, it was seen in 1 on day 9 (10%), in 5 on day 11 (50%), in 8 on day 15 (80%), and in all on day 17.

Histopathological study revealed that while the samples taken from the NS group showed that the skin surface was covered by a thin layer made up of necrotic fibrinoid debris (Fig.a); there was an inflammatory infiltration area abundant in active non-specific neutrophiles reaching to the subcutis beneath this layer. Granulation tissue and fibrous collagenous scar tissue distinct on the ulcer borders were observed under the inflammatory infiltration area (Fig.b). It was observed that ulceration and inflammation were localized in the subepithelial field in the skin cross-sections of the insulin group and that there was a significant increase in collagen bundles. Moreover, it was seen that the fibrous collagenous scar tissue was so increased that it filled up the whole crater in the insulin group (Fig.c and d). Further, it was observed that the epidermis was thicker in insulin applied wounds.

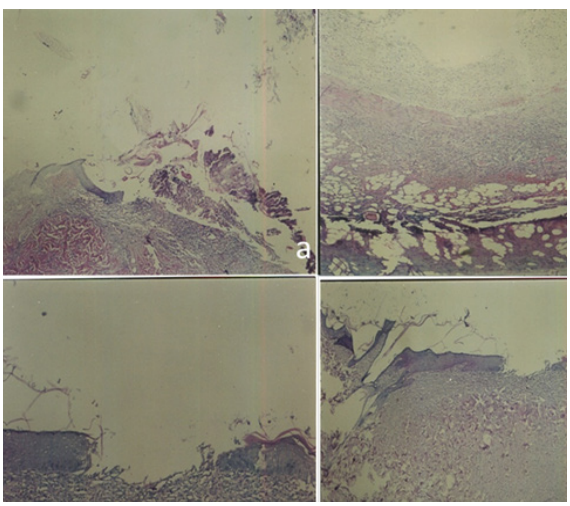

***Figure**. Immunohistochemical outlook of the samples taken on day 20 of wound healing in normal saline and insulin groups. a and b (NS group): Normal neutrophile activation and normal collagen structure. c and d (Insulin group): Intensive collagen formation and intensive neutrophile activation.*

DISCUSSION

Wound healing is a complex and dynamic process whereby cellular structures and tissue layers are reconstructed. Adult human wound healing can be categorized into three stages: inflammatory phase, proliferative phase, and remodeling phase. Blood cells like macrophages and neutrophiles, extracellular matrix and mediators, various proteins, and some genes play a role in these phases. The perfect execution of all the three stages results in perfect wound healing.

The effect mechanism of insulin on wound healing is yet to be known. There are studies in literature regarding the effect of insulin on cutaneous wounds. Insulin triggers keratinocyte migration depending on dose and time in chronic wounds. Insulin demonstrates its effect through an insulin receptor dependent but EGF/EGF-R non-dependent way. The fact that it increases keratinocyte migration on the PI3K-Akt-Rac1 pathway and stimulates keratinocytes by enabling the production of α3 and LN332 molecules has been proven by in vitro studies (3-6). Insulin is the hormone that affects collagen production. Insulin selectively and strongly stimulates collagen production in skin fibroblasts (16, 17). Our study demonstrated that there was an intensive increase in organized collagen fibrils on the epidermis and dermis layers as a result of immunohistochemical staining of samples taken from the wounds of the insulin administered rats. We also established that the ulcer depth was much less in all the insulin administered rats than the NS rats as revealed by histological evaluation.

Insulin is one of the hormonal mediators taking part in collagen production. It is known that insulin selectively and strongly stimulates collagen production in skin fibro-

blasts (16, 17). Insulin takes part in all phases of wound healing through enhancing the extracellular matrix and collagen synthesis by increasing the proliferation of myofibroblasts during the fibroplasia stage, by elevating the macrophage activation through some ways in the inflammatory phase, and by increasing keratinocyte differentiation, migration, and proliferation in the re-epithelial stage (5, 6). Thus, reasons such as decrease in growth factor production, angiogenesis, macrophage functions, collagen accumulation, epidermal barrier function, keratinocyte and fibroblast migration and proliferation contribute to defective wound healing in diabetic patients (18-20). Lima et al. (21) stated that the use of topical insulin cream accelerated wound healing by activating some insulin signal pathways in rats with experimental diabetes in their double-blind placebo controlled study. Chen et al. (22) proved that insulin increased macrophage activations in their in vitro study. Thus, it is stated that insulin displays its effect during the acute phase by enabling the removal of necrotic tissues in the wound bed and preventing bacterial contamination. Werner et al. (23) argued that IGF-I played a more major role in wound healing in skin wounds formed in genetically diabetic rats and put forward that defective wound healing in genetically diabetic rats was related to decreased IGF-I levels. Liu et al. (24) showed that insulin was not only effective in re-epithelization but also in granulation tissue formation since it stimulated keratinocyte functions. We aimed to compare the effect of insulin on the pace of wound healing and quality to NS in our study. The results of our study revealed that the rate of wound closing and the period of complete epithelization in insulin applied wounds were distinctively shorter than the NS group. Moreover, we also observed that through the topical application of insulin there became a pronounced increase in collagen bundles and regular collagen strings in wound healing immunohistochemically.

NS is very frequently used in clinical practice for the treatment of chronic wounds. It contributes to the prevention of disinfection and contamination of the wound in the acute phase and to the formation of regular granulation tissues in the chronic phase. We firstly prefer NS for especially the treatment of infected wounds at our clinic. Further, we also believe that NS used in open wound healing both affects the prevention of contamination and the formation of granulation tissues. In our study, we evaluated the effects of insulin on wound healing in comparison to NS. We observed that the insulin group had a distinctively shorter period of wound healing and had a more quality wound healing process histopathologically than the NS group.

Consequently, wound healing was faster and better in the insulin group macroscopically and histopathologically.

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Conflict of Interest Statement

We authors declare no conflict of interest.

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