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Original Article

EVALUATION OF BURN WOUND HEALING ACTIVITY OF TOPICAL REGULAR INSULIN IN NON-DIABETIC AND STREPTOZOCIN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: The role of insulin in the regulation of energy metabolism, protein synthesis, cell differentiation and growth suggests that this hormone could also play an essential role in regulation of wound healing. Consequently, the aim of the present study was to investigate the effects of topical insulin administration on burn wound healing in both non-diabetic and streptozocin-induced diabetic Wistar rats.

Methods: Wound healing activity was assessed by burn-wound model. This study was conducted using six groups of Wistar strain adult rats of either sex (n = 6). First three groups were non-diabetic (ND) rats and the other three had diabetic (D) rats: (i) ND control (sterile water); (ii) ND standard (silver sulfadiazine cream); (iii) ND test (topical Insulin); (iv) D control (sterile water); (v) D standard (silver sulfadiazine cream); (vi) D test (topical insulin). Wound healing was assessed by wound contraction rate and complete epithelialization time.

Results: There was significant ($p<0.05$) delay in wound healing in diabetic rats when compared to normal rats. It was found that topical insulin administration enhanced burn wound healing by shortening the time needed for complete epithelialization in the non-diabetic and diabetic group.

Conclusion: This study revealed that topical insulin application to partial thickness burn wounds accelerates wound healing in rats with or without acute diabetes.

Keywords: Topical insulin, Diabetes, Burn wound healing, Streptozocin.

INTRODUCTION

Ever since man discovered fire he has burnt himself either accidentally or intentionally. Burns are the oldest type of injuries that man suffers from and it is one of the important causes of mortality and morbidity. Burns afflict all segments of the society, the rich, the poor, men and women, children and old may fall victim to it [1].

Depending on the area affected and the degree of severity, the burn victim may experience a wide number of potentially fatal complications, including shock, infection, electrolyte imbalance and respiratory distress. Beyond physical complications, burns can also result in severe psychological and emotional distress due to scarring and deformity.

Burn wound healing is a complex phenomenon that results in the restoration of anatomic continuity and function, accomplished by several processes which involve different phases including inflammation, granulation, fibro genesis, neo-vascularization, wound contraction and epithelialization [2]. Effective management of the burn wound requires understanding of the normal repair process, factors that affect healing, wound assessment and selection of intervention designed to optimize process of healing. Attempts have been made to accelerate wound healing either when it is progressing normally [3, 4] or when it is suppressed by various agents like corticosteroids [5] or when it is suppressed in conditions like diabetes mellitus [6].

Diabetes is a condition known even in its early stages to impair the normal course of wound healing, thus leading to chronic wounds. The wound-healing impairment in diabetes can be attributed to several factors including inadequate blood supply, decreased proliferative potential of fibroblasts, and decreased inflammatory changes [6].

The ability of insulin to regulate energy metabolism, protein synthesis, cell differentiation, and growth suggests an important role for this hormone in the regulation of wound healing. Studies using topical application of insulin to wounds are conflicting. Topical insulin improved healing in chronic foot ulcers in both diabetic and non-diabetic mice [7] nd prevented steroid-impaired corneal wound healing [8]. However, topical insulin therapy failed to improve healing of decubitus ulcers [9]. In experimental wounds, insulin appears to act synergistically with platelet-derived growth factor or epidermal growth factor (EGF) to increase granulation tissue and collagen deposition [10, 11]. However, the exact mechanism for this action of insulin is unknown. The aberrations in wound healing caused by experimental diabetes that insulin treatment obviates include decreased granulation tissue formation [12] and increased collagens and protease activity [11]. This study was aimed to investigate the effects of topical insulin administration on burn wound healing in both non-diabetic and streptozocin-induced diabetic Wistar rats.

MATERIALS AND METHODS

Animals

Adult Wistar albino rats of either sex weighing 150-250 g were housed in separate polypropylene cages, maintained under standard conditions with temperature (22–24^{0}C), 12-h light/12-h dark cycle and relative air humidity 40–60%. The animals were acclimatized to the laboratory conditions for one week before the start of the experiment. The animals were provided with a normal pellet diet (Amrit Feeds Ltd., Pune, India) and water ad libitum. Animals described as fasted were deprived of food for 16-h but had allowed free access to water. The experimental protocol was approved by the Institutional Animal Ethics Committee and experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines.

Induction of diabetes mellitus

After 12-h of fasting, diabetes mellitus was induced in the animals of group IV, V and VI by intraperitoneal (*i.p*) injection, of streptozocin

dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 30 mg/kg and maintained on ice prior to use. The animals were allowed to drink 10% glucose solution overnight to overcome the drug induced hypoglycemia. After a week time for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (fasting blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the experiment.

Induction of burn-wound among experimental animals

All the experimental animals were anaesthetized with ketamine 80 mg/kg (*i.p*) and secured to operation table in its natural position. An impression was made on the dorsal thoracic central region 5 mm away from the ears by using a round seal of 300 mm^2 diameter[13].

Burn wound was created by pouring hot molten wax at 80°C into a metal cylinder placed on clean shaven area on the back of the rat. The metal cylinder had the following specifications: (a) 300 mm area of circular openings (b) Capacity to hold 4.6 g of wax.

On solidification of wax (8 min), the metal cylinder with wax adhered to skin was removed, which left distinctly demarked circular wounds of 300 mm^2. After this each animal was placed in a separate cage for full recovery from anaesthesia before being returned to holding rooms. No local or systemic chemotherapeutic agents were given. During the experimental period the animals were housed individually and resuscitation was done with 10% glucose water.

The physical attribute of healing viz. (wound closure) contraction which mainly contributes for wound closure was studied by tracing the raw wound area on the tracing paper on day 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and further till complete epithelialization occurred, the criterion for complete epithelialization being fall of scab without any raw wound area. Wound area was measured by retracing the wound on a millimeter graph paper.

Experimental design

In the experiment, 36 adult Wistar rats (18 normal and 18 diabetic surviving rats) of either sex were divided into six groups (n= 6 animals/group). The corresponding treatment was done till 19 days as follow- *Group I:* Burn-wound induced non-diabetic animals (negative control) received normal saline (1ml/kg/day),twice daily *Group II:* Burn-wound induced non-diabetic animals (positive control) received standard drug silver sulfadiazine cream topically on the wound area twice daily

Group III: Burn-wound induced non-diabetic animals (test) received test drug 0.1 U of regular insulin [14] infiltrated on edge of wound area, twice daily *Group IV:* Burn-wound induced diabetic animals (negative control) received normal saline (1ml/kg/day),twice daily *Group V:*Burn-wound induced diabetic animals (positive control) received standard drug silver sulfadiazine cream topically on the wound area, twice daily.

Group VI: Burn-wound induced diabetic animals (test) received test drug 0.1 U of regular insulin [14] infiltrated on edge of wound area, twice daily.

Wound contraction

Wound contraction was noted by the following progressive changes in wound area planimetrically, leaving the wounding day. Sizes of the wounds were traced on a transparent paper every alternate day by restricting the pose of the animal identical throughout the monitoring.

The tracings were then transferred to 1mm^2 graph sheet from which the wound surface area was evaluated. The evaluated wound surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound as the first day wound size (approximately 450mm^2) as 100%, by using the equation.

$$\%\ wound\ contraction = Initial\ wound\ size - Specific\ day\ wound\ size\ X100 / Initial\ wound\ size$$

Epithelialization period

It was monitored by noting the number of days required for the eschar to fall from the burn wound surface without leaving a raw wound behind.

Statistical analysis

Using Statistical Package for the Social Sciences (SPSS version 16.0; SPSS Inc., Chicago, USA), data were expressed as mean ± standard error of mean and analyzed by one way analysis of variance (ANOVA) followed by post hoc Tukey test. A level for $p \leq 0{\cdot}05$ was considered to be statistically significant.

Results

Effect on wound contraction among burn-wound induced non-diabetic and diabetic animals

15th day onwards, there was significant impact on wound contraction in burn-wound induced non-diabetic and diabetic animals treated with insulin topically in comparison with burn-wound induced control group(Table 1, 2). There was significant increase in percentage wound contraction on 19th day in burn-wound induced insulin treated diabetic animals when compared with burn-wound induced diabetic control animals. (Table 2). Topical insulin was found to increase wound contraction comparatively better than silver sulfadiazine cream.

Table 1: Effect of topical insulin on percentage wound contraction among burn wound induced non-diabetic animals

Days	Group I- Non-diabetic control	Group II- Positive control	Group III- Insulin
3rd	17.09±2.25	25.76±5.76	30.78±3.52
7th	41.12±6.07	40.08±4.08	44.01±3.10
11th	49.96±5.34	51.33±3.36	57.90±3.48
15th	66.81±4.85	76.52±1.81	90.21±3.54***
19th	84.55±2.76	92.72±1.59	97.10±2.50*

n = 6, number of rats in each group; Values are mentioned as mean ± standard error of the mean. Table 1 shows the percentage wound contraction of non-diabetic animals at different time intervals. ***indicates statistically significant difference compared with burn-wound induced non-diabetic control group on 19th day (p< 0.001);*indicates statistically significant compared with burn-wound induced non-diabetic control group on 19th day (p< 0.05).

Table 2-Effect of topical insulin on percentage wound contraction among burn-wound induced diabetic animals

Days	Group IV- Diabetic control	Group V- Positive control	Group VI- Insulin
3rd	9.96±1.62	18.43±5.12	20.47±5.59
7th	33.34±2.89	38.05±2.64	41.87±3.44
11th	53.05±3.59	51.12±3.73	47.32±3.63
15th	67.92±3.49	65.94±2.23	75.36±3.16
19th	80.50±2.31	86.53±3.43	96.90±3.09**

n = 6, number of rats in each group; Values are mentioned as mean ± standard error of the mean. Table 3 shows the percentage wound contraction of non-diabetic animals at different time intervals. **indicates statistically significant compared with burn-wound diabetic control group on 19th day (p< 0.01)

Effect on duration of epithelialization among burn-wound induced non-diabetic and diabetic animals:

Both silver sulfadiazine cream and topical insulin significantly attenuated the duration of epithelialization among burn-wound induced non-diabetic and diabetic treated animals in comparison with burn-wound induced non-diabetic and diabetic control (untreated) animals (Figure 1).

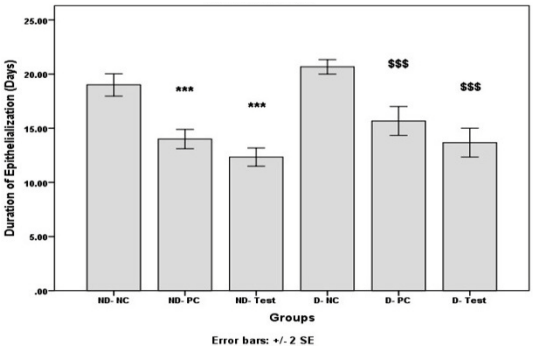

Fig. 1: Epithelialization period among burn-wound induced non-diabetic and diabetic animals

n = 6, number of rats in each group; Values are mentioned as mean ± standard error of the mean. Figure 1 show the duration of epithelialization among experimental animals. ND, non-diabetic; D, diabetic; NC, burn-wound induced negative control (untreated); PC, burn-wound induced positive control; Test, burn-wound induced animals treated with insulin topically. ***indicates statistically significant compared with non-diabetic control (p<0.001), $$$indicates statistically significant compared with diabetic control (p< 0.001).

DISCUSSION

Diabetes is one of the factors affecting the normal course of wound healing. Diabetic wound healing is characterized by a delay in cellular infiltration and formation of granulation tissue, and diabetic wounds have a prolonged epithelialization time[15]. Experimental diabetes was shown to impair wound healing by decreasing collagen concentration and formation of granulation tissue[12, 14, 16], and also by increasing activities of protease and collagenase [11]. Collagen provides tensile strength, organization and integrity to the connective tissues, and plays a role in hemostasis by interacting with thrombo cytes. Insulin is one of the potential hormonal mediators of altered collagen production. It is known to stimulate collagen synthesis in skin fibroblasts in a strong and selective manner [17]. One of the studies reported the presence of insulin receptors in keratinocytes of the epidermis and in hair follicles, and identified signaling pathways through which insulin can promote growth in the skin [15]. It was shown that human keratinocytes are dependent on insulin for their growth[18]. Several in vitro experiments suggest that growth factors such as insulin can act as chemo-attractants and mitogens for the cells involved in wound healing [19-21] and that growth factors can stimulate angiogenesis, extracellular matrix formation and degradation, and cytokine release[19].

This data summated suggests that in diabetic rats healing was impaired, and there was significant (p<0.05) delay in wound healing; the mean period of epithelialization in normal control group was 19.00 ± 0.51 days whereas in diabetic control group it was 20.66 ± 0.33 days.

It has been suggested that alterations in the wound-healing process are present even at the onset of diabetes and that early diabetes can be associated with deficiencies in the defense cells involved in normal wound healing [22] and also with a marked decrease in the production of collagen[23].

The results of the present study showed that the median time of complete epithelialization was 11.5 days (range 9–13) for the nondiabetic controls, and 8 days (range 7–10) for the nondiabetic rats receiving topical insulin (p<0.001). Since the study was done using incision wound model where chances of infection are less so we conducted a study on a burn wound model in diabetic rats which poses a greater threat to survival as the chances of infection are high. The most recent study on the effects of topical insulin in wound healing was conducted on human skin cell cultures [24]. They explored the molecular impact of topical insulin on keratinocytes, the cells that regenerate the epidermis after wounding, and on microvascular endothelial cells, the cells that restore blood flow. Using various cell and molecular techniques, the researchers discovered that insulin stimulates human keratinocytes in culture to proliferate and migrate. In cultured human microvascular endothelial cells, the insulin stimulates only migration into the wound tissue [25].

The skin wounds of the rats treated topically with insulin healed faster, the surface cells in the epidermis covered the wound more quickly, and the cells in the dermis rebuilt blood vessels more rapidly [26]. It was also shown that topical insulin stimulated the proliferation and migration of keratinocytes and the migration of micro-vascular endothelial cells. [26] Our results also concur with those of a similar study in which topical insulin provided better rates of wound healing than treatment with base or control preparations[7], and one in which diabetic rats that received topical insulin alone or in combination with epidermal growth factor (EGF) had lower collagenase activity than both control and diabetic EGF groups[11]. Our results were not in accordance with those from the study of Grotendorst et al. [10], which suggested that insulin had no effect on the rate of new tissue formation. However, in that study, a combination of platelet-derived growth factor (PDGF) and insulin caused an even more rapid increase in collagen deposition than that found with PDGF alone. In conclusion, this study supports the notion that even in the early stages of diabetes; the wound-healing process is impaired, and revealed that topical insulin application to non-diabetic as well as acute diabetic cutaneous wounds accelerates wound healing in rats. The findings of our study could be used as an alternate to or in combination with currently used wound treatment for rapid and better healing.

CONFLICT OF INTERESTS

Declared None

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