Original Article

The Effect of Subcutaneous Unfractionated Heparin and Low-Molecular Weight Heparin toward Modification of Diabetic Acute Influence on Surgical Wound Healing in Rats

Shima Shafagh, Amirhasan Matini[1], Mahdi Noureddini[2], Mojtaba Sehat[3], Alireza Kalani, Majid Kalbasi Gharavi[4]

Departments of General Surgery, [1]Clinical Pathology, [2]Physiology, [3]Epidemiology, School of Medicine, Kashan University of Medical Sciences, Isfahan, [4]Department of General Surgery, Isfahan University of Medical Sciences, Isfahan,Iran

ORCID

Shima Shafigh: https://orcid.org/0000-0001-7229-3234
Amirhosein Matini: https://orcid.org/0000-0002-9106-0249
Mahdi Noureddini: https://orcid.org/0000-0001-5148-9211
Mojtaba Sehat: https://orcid.org/0000-0001-7142-4829
Alireza Kalani: https://orcid.org/0000-0003-1170-687X
Majid Kalbasi Gharavi: https://orcid.org/0000-0002-7833-2991

Abstract

Background and Objectives: Diabetes mellitus is one of the disturbing factors in surgical wound repair that recognizing these mechanisms, and modifying them can be useful in preventing surgical wound complications. The aim of this study is to assess the effect of unfractionated heparin (UH) and low-molecular weight heparin (LMWH) on diabetic wounds in the rats. **Materials and Methods:** This study as a clinical trial in the animal phase was done in two groups that each group consists of three subgroups containing eight rats in each. Initially, under anesthesia, the skin was incised surgically, and interventions with UH in the first subgroup of each group, LMWH in the second subgroup, and normal saline in the third subgroup were performed. In the first group on the 7th day and the second group on the 10th day, wound biopsy was taken and examined pathologically. Finally, data were analyzed using Kruskal–Wallis and one-way analysis of variance statically test. **Results:** The results of this study showed in every two groups, there was a statistically significant difference between the subgroups for fibroblast and vascularity status, but this difference was not significant for epithelialization and collagen level. In addition, wound length in both the groups had a significant difference between subgroups. **Conclusion:** In this study, there was a relative difference between the administration of UH and low-molecular weight and surgical wound healing in diabetic rats.

Keywords: Diabetes, heparin, surgical wound healing

Introduction

Skin is the largest single organ of the body, which plays an essential role as a protective barrier against the external environment. The breakdown of this integrity is known as wound, which, regardless of its mechanism, needs to be repaired or paid attention. Wound healing is starting as a preplanned process and is then completing gradually during different phases.[1] However, this process is not always well developed, and several disturbing factors such as hypoxia, infection, tobacco abuse, nonsteroidal anti-inflammatory drugs, vitamin deficiency, and metabolic diseases like diabetes mellitus inhibit the process of surgical wound healing.[2] Accordingly, diabetes is known as one of the most crucial factors related to the reduced essential products for repair, the impaired angiogenic response, the decreased macrophage

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Address for correspondence: Dr. Alireza Kalani, Department of General Surgery, School of Medicine, Kashan University of Medical Sciences, Isfahan, Iran.
E-mail: dr_kalani1364@yahoo.com

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function, the decreased collagen accumulation, the decreased granulation tissue quality, and the decreased epidermal nerve function.[3] Hence, modulation of diabetes and its associated pathologic mechanism on wound healing have been considered since a long time ago.

Although the most effective efforts are hyperglycemia correction and diabetes control, there are also some studies reporting that medications would improve healing process. For example, vitamin D has been proved to have a positive effect on healing diabetic ulcers in the animal phases due to its anti-inflammatory property.[4] Therefore, it is important to know the factors that can improve diabetic wound healing.

Heparin is an anticoagulant bind to the enzyme inhibitor antithrombin III (AT) and inactivates thrombin, factor Xa, and other proteases. The role of heparin in wound healing has been studied in both *in vitro* and *in vivo* researches and concluded several proved effects on endothelial cell repair, increase in capillary circulation, and decreased healing time.[5]

The aim of this study was to assess the effect of low-molecular weight heparin (LMWH) and unfractionated heparin (UH) on wound healing in diabetic rats.

Materials and Methods

This study was designed as a clinical trial to evaluate the effect of unfractionated and LMWH on the wound healing process in the background of diabetes mellitus in rats. In this study, we considered two groups as follows: the first group for evaluation on the 7th day and the second group for performing evaluation on the 10th day. Moreover, each group consisted of the following three subgroups: the first subgroup for intervention with UH, the second subgroup for intervention with LMWH, and the third subgroup was considered as the control group. The sample size was determined to be 24 in each group (8 in each subgroup) using equation mead. After being approved by the ethics committee, 48 male albino rats weighed between 250 and 300 g and aged between 8 and 12 weeks were selected. Throughout the study, appropriate laboratory conditions including the temperature 21°C ± 1°C, light-dark cycle as 12 h' light cycle and 12 h' dark cycle routinely, and adequate storage of water and food were obtained. A single dose of alloxan 120 mg/kg was injected intraperitoneally to induce diabetes mellitus, and the diagnosis was considered fasting blood glucose more than 150 mg/dl.

By passing 2 weeks from diabetes diagnosis, under anesthesia, a combination of intraperitoneal ketamine 60 mg/kg and xylazine 4 mg/kg, a 20-mm full-thickness skin was incised in the midline back of rats and then spent to heal through as secondary repair.

The pharmacological intervention was done by subcutaneous multiple injections of heparin 1000 U/kg in the first subgroup, enoxaparin 1 mg/kg in the second subgroup, and 0.5 ml normal saline in the control subgroup. The first dose was injected 12 h before incision, and the following doses were injected 6 h, 24 h, and daily up to 7 or 10 days (in each group) after incision. In the first group on the 7th day and in the second group on the 10th day, wound biopsies were taken [Figure 1]. Finally, death was induced in rats by a high-dose anesthesia injection.

For the biopsy, the wounds were extracted full thickened, and histologic slides were prepared, evaluated, and then scored as four criteria, including epidermal regeneration, collagen fibers, fibroblast level, and vascularity by a pathologist.

The results were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows software released 2015, version 23.0, Armonk, New York, USA. based on the distribution of continuous variables using analysis of variance test and Kruskal–Wallis statistical test for discrete random variables. The significance level was defined as <0.05.

Results

In pathological examination of the wound among subgroups on the 7th day, there was no significant relationship for epithelial remodeling (P = 0.877) and thickness of collagen fibers in the wound (P = 0.494). However, there was a significant relationship between fibroblast level (P = 0.013) and vascularity improvement (P = 0.045).

For the evaluation of wound on the 10th day, there was no significant relationship between epithelial remodeling (P = 0.187) and thickness of collagen fibers (P = 0.418). However, there was a significant relationship between fibroblast level (P = 0.014) and vascularity improvement (P = 0.034).

The mean length of wound according to groups and subgroups is indicated in Table 1, by one-way analysis of variance and multiple group comparisons, there was no significant

Table 1: The relationship between unfractionated heparin and low-molecular weight heparin and wound length

	Mean±SD	Multiple group comparisons	Significant*
Day 7			
UFH	1.26±0.19	LMWH	0.20
		NS	0.08
LMWH	1.39±0.11	UFH	0.20
		NS	0.702
NS	1.41±0.08	UFH	0.08
		LMWH	0.702
Day 10			
UFH	0.4±0.07	LMWH	0.19
		NS	0.00
LMWH	0.26±0.26	UFH	0.19
		NS	0.00
NS	0.88±0.37	UFH	000
		LMWH	0.00

*One-way ANOVA, *Post hoc* LSD. LMWH: Low-molecular weight heparin, UFH: Unfractionated heparin, SD: Standard deviation, NS: Normal saline, ANOVA: Analysis of variance, LSD: Least significant difference

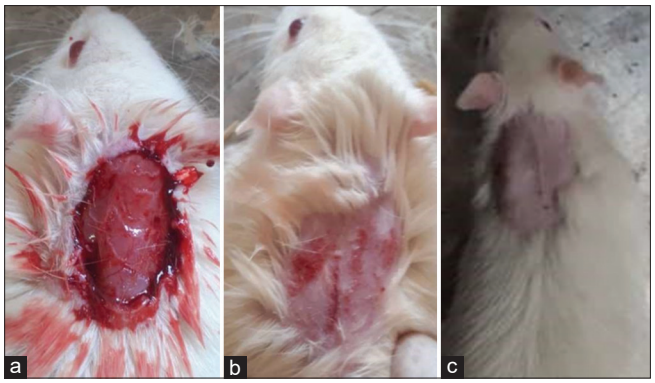
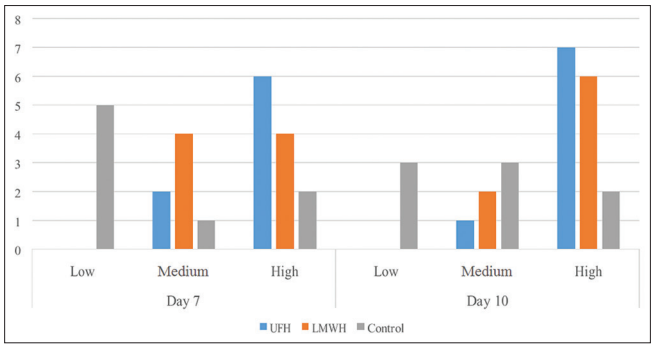
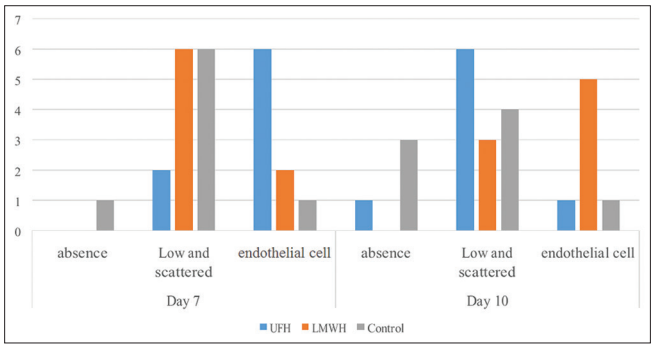


Figure 1: Sample of studied rats. (a) The 1st day of intervention by unfractionated heparin. (b) 7th day in intervention by unfractionated heparin. (c) 10th day in intervention by unfractionated heparin



Graph 1: Fibroblasts level on days 7 and 10



Graph 2: Vascularity on days 7 and 10

relationship between reduction of wound length in 7th day and 10th day.

Discussion

The wound healing process involves several sequences of cellular and molecular processes,[6] in which the primary induction process by immune cell aggregation is important. Macrophages are immune cells that are essential for healing,[7] and their absence causes a delay in epithelialization,[8] the decreased collagen accumulation,[9] impairment in angiogenesis,[10] and the reduced cell proliferation. In addition, epithelialization is an essential step in the wound healing process, and it must be emphasized that wound healing cannot be done in the absence of epithelialization, while it can be disrupted in chronic wounds.[11] Therefore, for studying wound healing, different checkpoints can be considered for that and its ongoing processes, including epithelialization, collagen fibers formation, and fibroblast levels in the wound environment.

Many *factors affect wound healing, of them, the role of* diabetes is prominent. Immune cells such as polymorphonuclear leukocytes and fibroblasts are involved in wound healing influenced by diabetes. On the other hand, hyperglycemia reduces the available insulin and increases insulin resistance, which consequently reduce the cellular response to tissue damage.

In diabetes, wound healing impairment is affected by numerous changeable or unchangeable factors such as epidermal nerve loss[12] or angiogenic disorders.[13] Although cellular and molecular mechanisms leading to wound repair impairment are not well understood yet, and therapeutic approaches are limited in this regard, efforts to improve these mechanisms have been continued.[14]

Heparin is a probable agent that may reverse the changeable effect of diabetes on wound healing, but it needs precise investigation to be confirmed. In the present study, the effects of two types of heparin were assessed using the above-mentioned checkpoints.

Although heparin is routinely prescribed to prevent venous thrombosis after different surgeries, its effect on the wound still remains controversial. In Oken *et al*.'s study, administration of enoxaparin was shown to be associated with a delay in the wound healing process, which was due to a decrease in inflammatory status and fibroblast cells in the acute phase of the wound.[15] However, Galvan found that its administration in diabetes, due to the proliferation of endothelial cells and the improvement of microcirculatory circulation, reduces the duration of wound healing.[16]

The results of the present study among subgroups show that there was no significant relationship between epidermal regeneration and collagen fibers thickness on days 7 and 10, while there was a relationship between fibroblast cell level and vascular remodeling [Graphs 1 and 2]. Oken *et al*. revealed the role of heparin in the delayed epithelialization on the 15th day, as well as the adverse effects of heparin or warfarin on wound healing.[15] However, in this study, there was no relationship between epithelialization and heparin administration on days 7 and 10.

Black *et al*. in their study performed in the background of type 1 diabetes, as histologically, there was always a decrease in collagen due to the fibroblasts' slow response, and this situation was markedly improved by controlling hyperglycemia.[17] Overall, in diabetes mellitus, it was shown that what is impaired is the fibroblast response that results in collagen synthesis and according to our finding, there was no significant association between heparin administration and collagen improvement. However, heparin plays a positive role

in the growth of fibroblast cells. Although it was expected that the repair process following degradation in primary function would be disrupted by heparin, in some studies, it has been observed that the number of fibroblasts eventually increases, and the collagen fibers become more regular.[18]

In this study, both heparin and enoxaparin administrations were associated with a significant improvement in the vascular bed of wound tissues, which was also better for UH. In comparison with the Jörneskog *et al*.'s study, administration of heparin has been shown to be associated with improved microvascular levels in chronic diabetic wounds.[19] In contrast, in a study by Oturai, heparin had no effect on diabetes-induced vasculopathies.[20]

Wound length evaluation was another aim of this study, and administration of heparin coinciding by reducing the wound's length by 10, of course, the effect of heparin, was more than enoxaparin, which is comparable to the Kweon *et al*. study, which they found after 2-week administration of topical heparin in the case group, there was almost complete repair, increase in thickness, and skin tissue at the wound site.[21] However, in Civelek *et al*. study, administration of low-molecular weight heparin had a harmed effect on the wound.[18]

Durmaz *et al*. study was in opposition to the results of the present study, and there was a statistically significant difference between groups on 7th and 10th-days in wound healing, and this finding showed that heparin had a positive effect on wound healing over a maximum period of 10 days.[22]

Conclusion

This study in the animal phase revealed that the administration of UH or low-molecular weight heparin, as a modifier agent, can improve acute wound healing in diabetes background. However, neither UH nor low-molecular weight heparin did not reduce wound length in the 7th or 10th day. Despite these results, the definite effect of heparin on wound healing improvement is still unclear, particularly in the human model, so it requires more detailed and larger volume studies.

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Conflicts of interest

There are no conflicts of interest.

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