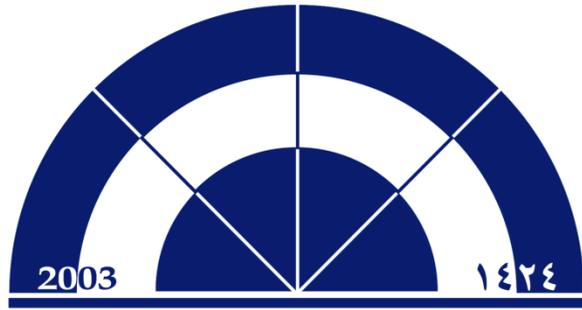


A Study Of Wound Healing By Local Injection of Insulin

دراسة عن التئام الجروح بحقنة أنسولين موضعية

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Abstract :

Objectives: In this prospective study we aim to demonstrate the effect of small doses of topical long acting insulin (zink insulin) application in wound healing.

Methods: Thirty eight patients were studied in our private out-patient clinic between the years 2009 to 2011 , long acting insulin (zinc insulin) was used to avoid the hypoglycemic effect of insulin .

Results: From a total of 38 patients, 20(52.6%) were males and 18(47.4%) were females. The median age was 49 years with a mean age of 35.11 ± 11.543 years. There were different causes of the wounds, 21(55.3%) patients had previous operations leading to the wound, 9(23.7%) developed chronic wounds after burns, and 8(21.0%) had a wound due to trauma. The wounds were distributed in different sites of the body. In the trunk 24(63.2%) patients, in the extremities 11(28.9%) patients and in the breast 3(7.9%). All the wounds were previously dressed with saline. The sizes of the wounds varied, minimum wound size was 1cm and maximum was 16cm

with a mean of 6.32 ± 4.319 cm. 13(34.2%) of the wounds were infected, 18(47.4%) had allergy to stitches , 4(10.5%) had unhealthy granulation tissue and 3(7.9%) were covered with tough fibrin. These wounds were prepared prior to the study by giving antibiotic, removing the stitches, excising bad granulation tissue and removing fibrin. Soluble Insulin injection was prepared at a concentration of 1-Unit/10ml distilled water, then injected superficially in the inside of the wounds of the patient, and the effect was observed. The time range of saline-insulin treated wound closure was 15 days with a mean of 7.13 ± 4.461 days. All patients had an excellent outcome and their wounds closed in a short time without any complications.

Conclusion: Topical insulin application leads to acceleration of wound healing. The duration of wound healing and outcomes in our study were excellent. This depends on many factors also which should be taken seriously like wound infection of the wound and size.

Keywords: Insulin, diabetes, topical insulin, neutrophils, MIP-2, anti-neutrophil antibodies, wound healing,

Objectives: To study the effects of small doses of long acting (zinc insulin) injected locally in wound healing.

الملخص :

بنسبة (٢١.٠%)، وفي الجسد بنسبة (٦٣.٢%)، وفي الأطراف بنسبة (٢٨.٩%) . وفي الثدي بنسبة (٧.٩%) . جميع هذه الجروح كانت تضمد بماء الملح واليود. وتفاوتت فترات التضميد بين يومين إلى ١١٣ يوماً. حجم أصغر جرح كان ١ سم، وحجم أكبر جرح ١٦ سم . (٣٤.٢%) (١٣ من هذه الجروح كانت ملتهبة، و(٤٧.٤%) كانت مصابة بتحسس من الخيوط الجراحية، و(١٠.٥%) كان فيها تضخم نسيجي، و(٧.٩%) كانت مغطاة بطبقة فيبرين. تم حقن الجروح بحقنة الأنسولين بتركيز ١ وحدة من الأنسولين مذاب في ١٠ سم مكعب ماء مقطر. استغرق التئام الجروح الفترة ما بين يوم واحد إلى ١٦ يوماً، التأم جميع الجروح في فترات بسيطة دون أي مضاعفات.

الأهداف: أهداف هذا البحث هي شرح كيفية عملية سرعة التئام الجروح باستعمال حقنة أنسولين طويل الأمد موضعية على الجرح .

المنهجية: أجري هذا البحث على ٣٨ مريضاً يزورون عيادتنا الخاصة خلال الأعوام ٢٠٠٩ إلى ٢٠١١. وقبل البدء بحقن الأنسولين قمنا بتهيئة الجروح، وذلك بعلاج الالتهابات والتحسس وغيرها من مشاكل الجروح. أستعمل أنسولين طويل الأمد وذلك تجنباً لهبوط مفاجئ للسكر .

النتائج: تم حقن مادة أنسولين طويل الأمد موضعياً داخل الجروح لـ ٣٨ مريضاً، منهم ٢٠ ذكراً و ١٨ أنثى. تفاوتت أعمارهم بين ١٧ و ٦٦ عاماً. كان (٥٥.٣%) جرحاً نتيجة عمليات جراحية سابقة، ونتيجة حروق كانت بنسبة (٢٣.٧%)، ونتيجة حوادث

الكلمات المفتاحية: أنسولين طويل الأمد، مرض السكري، أنسولين موضعي، نوتروفيل، أم أي بي ٢-، أجسام ضد النوتروفيل، التئام الجروح .

Introduction :

In the early 20th century, insulin was first used to treat diseases other than diabetes.^(1;2) various animal models showed that systemic insulin treatment accelerated healing from fractures, skin cuts, and skin ulcers.^(3;4;5;6) Low-dose topical insulin stimulated migration of keratinocytes and vascular endothelial cells through the insulin receptor-mediated pi3k-akt-*rac1* signal pathway. These events promoted re-epithelialization and wound healing.^(7;8)

It has been reported that insulin regulates systemic inflammatory responses, the cellular functions of neutrophils in the wound area. Low-dose topical insulin application decreases wound neutrophil infiltration and advanced wound neutrophil attenuation,

Neutrophils are the main type of cells that are involved in the inflammatory response. They clean exogenous pathogens through phagocytosis and release enzymes and Reactive Oxygen Species (ROS) to kill bacteria and other intruders. Macrophages also have the function of phagocytosis , therefore neutrophils are not essential to wound healing, since it has been shown that anti-neutrophil antibodies do not interfere with healing.⁽⁹⁾ On the contrary, depletion of neutrophils facilitates wound healing and improves the quality of recovery.⁽¹⁰⁾ Additionally, prolonged neutrophil infiltration may contribute to impaired wound healing. ⁽¹¹⁾

MIP-2, a member of the CXC chemokines family, strongly induces neutrophil chemotaxis.⁽¹²⁾ With topical insulin application, traumatic MIP-2 expression significantly decreases. Similarly with the change of MIP-2, wound neutrophils notably decreases. These observations suggest that topical insulin regulates the inflammatory response in the wounded area by restraining wound neutrophil infiltration through inhibition of chemokine MIP-2 expression. Vascular permeability also regulates inflammatory cell recruitment. Despite regulating MIP-2 expression, insulin stabilizes vessel endothelial barrier function. ⁽¹³⁾

It has been reported that diabetic wounds have elevated neutrophils and prolonged neutrophil infiltration.⁽¹³⁾ Conversely, neutrophils in wounds of patients with diabetes are dysfunctional in

terms of phagocytosis, migration, and bactericidal actions.⁽¹⁴⁾The present findings of insulin-induced stimulation of neutrophil functions may help advance the clinical treatment of chronic, nonhealing diabetic wounds.

Patients and Methods :

Thirty eight patients suffering from acute and chronic wounds as a result of various types of trauma (surgical and accidental) were collected from our private out-patient clinic during the years 2009-2011. They were randomly selected to receive local insulin injection directly in to the wounds during their wound dressings. The chronic wounds could not close inspite of the previous dressings. Acute wounds which were recently acquired were taken into the study too. Patients with diabetes mellitus were excluded for fear of interference with their normal regimen, otherwise all other types of patients were accepted.

Each patient was dealt with independently. A clinical record was created for each patient. Full history was taken and physical examination was performed and the wounds were carefully inspected and all positive findings were recorded. Any pathological conditions in the wounds were treated and the wounds were prepared in advance before the commencement of the study. Infections were treated by appropriate antibiotics, allergic surgical stitches were extracted, unhealthy granulation tissue was excised and fibrin was removed.

To all these patients a peripheral IV line was established for emergency purposes and they were asked to take a heavy meal before comming. Just before starting the study a series of procedures were performed.

- 1- A concentrated glucose solution (40%) was injected intravenously.
- 2- The wounds were cleaned with physiological solution.
- 3- Long acting insulin (zinc insulin) was used for this study (soluble insulin was not used to avoid sudden hypoglycemia).

Insulin solution was prepared at a concentration of 1.0-Unit of insulin dissolved in 10cc distilled water. 4-This solution was injected evenly throughout the inside of the wound, and this concentration was maintained for all patients throughout the study. 5-A sterile dry gauze was applied 6-and the wounds were dressed. These steps were done daily for each patient.

Results :

The study was done on 38 patients 20(52.6%) were males and 18(47.4%) females.(Table 1).

Table 1: Patients distribution according to sex

Sex	No.	%
Females	18	47.4
Males	20	52.6
Total	38	100.0

The ages of the patients were between 17 years and 66 years, with a mean age of 35.11 ± 11.543 . median was 49.

Wounds due to operations accounted for more than 50% of the patients, i.e. 21(55.3%) while burns and trauma accounted for 9(23.7%) and 8(21.0%) respectively. (Table2).

Table 2: Distribution of the patients according to the causes of the wounds

Causes of the wounds	No.	%
Operations	21	55.3
Burn	9	23.7
Trauma	8	21.0
Total	38	100

Most of the wounds were in the trunk 24(63.2%) patients, followed by the extremities 11(28.9%) patients, then in the breast 3(7.9%).(Table3).

Table 3: Distribution of the patients according to the sites affected

Sites of the wounds	No.	%
Trunk	24	63.2
Extremities	11	28.9
Breast	3	7.9
Total	38	100

All patients were managed previously i.e. after their first insult, by the usual classical dressings with saline and iodine, some of the patients were submitted to various surgical interventions on the same area of the wound.

The wounds varied in size. between 1 to 16 cm. With a mean size of 6.32 ± 4.319 cm. The smallest wound was as a result of deep burns and grossly thick fibrosis was impairing its closure.

Nearly half of the patients 18(47.4%) had stitch allergy while 13(34.2%) of the wounds were infected. The infected wounds had been treated for a long time with local antibiotics without improvement. On examination they were covered by thick fibrosis wall. 4(10.5%) had unhealthy hypergranulation tissue preventing tissue advance and 3(7.9%) had tough fibrin covering the wound and hindering it from closure. (Table5).

Table5: Distribution of the patients according to the clinical condition of the wounds

Type of wound	No.	%
Infected	13	34.2
Stitch allergy	18	47.4
Granulation tissue	4	10.5
Tough fibrin	3	7.9
Total	38	100

The stitches were causing distortion of the wound and even after stitch removal the wound was still distorted and deprived of blood supply which was hindering their proper closure.

A solution of 1.0-Unit of soluble insulin dissolved in 10cc of distilled water was used as local injections. The average time of wound closure treated with saline and insulin was 15 days, with a range of 1 day and to 16 days and a mean of 7.13 ± 4.461 days (Table6).

Table 6: Time of closure

Statistical discription	No.	Average	Minimum	Maximum	Mean	Std.Deviation
Age of patients	38	49	17	66	35.11	11.543
Size of wounds	38	15	1	16	6.32	4.319
Closure time in days for saline-insulin treated	38	15	1	16	7.13	4.461

The prognosis of all the 38 patients was excellent, they showed accelerated and healthy closure of the wounds (Table 7).

Table 7 : Prognosis

Prognosis	No.	%
Excellent	38	100
Bad	0	0
Total	38	100

Discussion :

Insulin is a hormone known primarily for regulating sugar levels in the blood but recently researchers at the University of California, Riverside, found that applying insulin directly to skin wounds significantly enhanced the healing process.

Wound healing is divided into 3 sequential, overlapping, phases: inflammatory, proliferation, and remodeling. In the early 20th century, insulin was first used to treat diseases other than diabetes.⁽¹⁵⁾

It has been reported that insulin regulates systemic inflammatory responses, whereas the regulation of traumatic inflammation by topical insulin has not been studied.⁽¹⁶⁾ Liu Y and Zhang XJ, et al. reported in their study that low-dose topical insulin application also promoted healing of thermal traumas in rats and incision wounds in rabbits.^(17,18) Local injection of small dose of insulin may accelerate burn wound healing due to its role in promoting the proliferation and division of the repairing cells⁽¹⁹⁾

Low-dose topical insulin stimulates migration of keratinocytes and vascular endothelial cells through the insulin receptor-mediated PI3K-Akt-Rac1 signal pathway. These molecular events could trigger re-epithelialization and angiogenesis, and hence, promote wound healing.^(20,21)

The present study showed that low-dose topical insulin application increased the rate of wound healing among the studied patients.

The age range our patients was wide from 17 years to 66 years, but no age group showed more rapid wound healing over the other. Both sexes and all ages showed equally accelerated healings.

The most common cause of the wounds in our study was post-operative. Wound infection coupled with allergy to suture materials used in our hospitals. The other cause was burns secondary to gas explosion which is common in our set up. Wounds secondary to trauma were few. (Table2).

Some wounds were acute and others chronic. The periods of the previous dressings in the patients in our study were variable, starting from two days after the primary insult- in patients with trauma - up to more than 3.5 months in patients with operations and burns. "Xuelian Chen, MD, et al. " published a study on topical use of insulin to accelerate wound healing on recent self made wounds on mice.⁽²²⁾

All patients in our study got primarily the same usual classical dressing i.e. saline and iodine dressings, before arriving to our care.

Our patients showed varying sizes of the wounds. From 1cm. to 16cm. with a mean size of 6.32 ± 4.319 cm.

At the examination of the wounds, nearly half of the wounds had allergy to the nonabsorbed suture material with stitch infection. This was true in 18(47.4%) patients who were operated previously. This may be attributed to the high affinity that our patients exhibit towards developing allergy to surgical sutures. A great number of our patients had postoperative wound infection 13(34.2%), which reflects the high rate of postoperative infections. Unhealthy granulation tissue occupied the third place among the causes of open wounds in our patients 4(10.5%), a fact commonly seen in our

practice due to the improper management of the wounds and neglect of therapy in most patients, while 3(7.5%) had tough fibrin, probably due to insufficient wound care in the patients. The last two arrest the skin from progressing. All these were important factors that kept the wounds open.

In the year 2012 in Fudan University (Shanghai, China), Xuelian Chen, MD, et al. used 0.03-U insulin dissolved in 20- μ L saline to inject mice for the purpose of their wound healing.⁽²²⁾ In another study on animals in (1983), Hanam SR, Singleton CE, et al. have reported that insulin at a concentration of 0.5 U/100g gives best effect on wound healing.⁽²³⁾ In our study we used 1.0-Unit of soluble insulin diluted in 10cc. distilled water. This is a concentration little higher than that reported in studies in other countries, but it did give us excellent results without complications. To avoid any hypoglycemic effect we injected the patients with 40% glucose solution prior to insulin injection each day.

Xuelian Chen, MD, et al. ⁽²²⁾ recorded a mean time of wound closure of 6.67 ± 0.52 days, which was significantly shorter than that of the saline treated wounds 8.17 ± 0.75 days in their study; and a $P < 0.05$. In our patients the wounds had a mean time of closure of 7.13 ± 4.461 days.

All patients who were treated with insulin got complete and accelerated wound healing.

Conclusion :

Topical insulin application leads to acceleration of wound healing. The duration of wound healing and outcomes in our study were excellent. This depends on many factors also which should be taken seriously like wound infection of the wound and size.

References :

1. King L, Kennaway EL, Piney A. A note on the action of insulin in normal persons. *J Physiol.* 1928;66:400-402.
2. Joseph B. Insulin in the treatment of non-diabetic bed sores. *Ann Surg.* 1930;92:318-319.
3. Gregory WB Jr. Effect of insulin on the healing of bone wounds in albino rats. *J Dent Res.* 1965;44:487-492.
4. Rosenthal SP. Acceleration of primary wound healing by insulin. *Arch Surg.* 1968;96:53-55.
5. Hanam SR, Singleton CE, Rudek W. The effect of topical insulin on infected cutaneous ulcerations in diabetic and nondiabetic mice. *J Foot Surg.* 1983;22:298-301.
6. Hawkins HK, Nguyen TT, Sakurai Y, et al. Effects of insulin on wound healing. *J Trauma.* 1998;44:342-345.
7. Liu Y, Petreaca M, Martins-Green M. Cell and molecular mechanisms of insulin-induced angiogenesis. *J Cell Mol Med.* 2009;13(11-12):4492-4504.
8. Liu Y, Petreaca M, Yao M, Martins-Green M. Cell and molecular mechanisms of keratinocyte function stimulated by insulin during wound healing. *BMC Cell Biol.* 2009;12(10):1.
9. Simpson DM, Ross R. The neutrophilic leukocyte in wound repair. *J Clin Invest.* 1972;51(8):2009–2023.
10. Dovi JV, He LK, DiPietro LA. Accelerated wound closure in neutrophil-depleted mice. *J Leukoc Biol.* 2003;73(4):448–55.
11. Wetzler C, Kämpfer H, Stallmeyer B, Pfeilschifter J, Frank S. Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: prolonged persistence of neutrophils and macrophages during the late phase of repair. *J Invest Dermatol.* 2000;115(2):245–253.
12. Wolpe SD, Sherry B, Juers D, Davatelis G, Yurt RW, Cerami A. Identification and characterization of macrophage inflammatory protein 2. *Proc Natl Acad Sci USA.* 1989;86(2):612–669
13. Gündüz D, Thom J, Hussain I, et al. Insulin stabilizes microvascular endothelial barrier function via

- phosphatidylinositol 3-kinase/Akt-mediated Rac1 activation. *Arterioscler Thromb Vasc Biol.* 2010;30(6):1237–1245.
14. Masuda M, Markami T, Egawa H, Murata K. Decreased fluidity of polymorphonuclear leukocyte membrane in streptozoc-induced diabetic rats. *Diabetes.* 1990;39:466–470.
 15. King L, Kennaway EL, Piney A. A note on the action of insulin in normal persons. *J Physiol.* 1928;66:400–402.
 16. Joseph B. Insulin in the treatment of non-diabetic bed sores. *Ann Surg.*
 1. 1930;92:318–319.
 17. Jeschke MG, Einspanier R, Klein D, Jauch K-W. Insulin attenuates the systemic inflammatory response. *Mol Med.* 2002;8(8):443–450.
 18. Liu Y, Zhang X, Zhang Z, Fang PY, Xu WS. Effects of topical application of insulin on the wound healing in scalded rats. *Zhonghua Shao Shang Za Zhi.* 2004;20:98–101.
 19. Zhang XJ, Wu X, Wolf SE, et al. Local insulin-zinc injection accelerates skin donor site wound healing. *J Surg Res.* 2007;142:90–96.
 20. *Zhonghua Shao Shang Za Zhi.* 2004 Apr;20(2):98-101
 21. Liu Y, Petreaca M, Martins-Green M. Cell and molecular mechanisms of insulin-induced angiogenesis. *J Cell Mol Med.* 2009;13(11–12):4492–4504.
 22. Xuelian Chen, MD, Xiong Zhang, MD, PhD, Yan Liu, MD, PhD: Wounds. 2012;24(7):178-184
 23. Hanam SR, Singleton CE, Rudek W (1983) The effect of topical insulin on infected cutaneous ulcerations in diabetic and nondiabetic mice. *J Foot Surg* 22: 298–301. 24. Xuelian Chen, MD, Xiong Zhang, MD, PhD, Yan Liu, MD, PhD: Wounds. 2012;24(7):17