Local Use of Insulin in Wounds of Diabetic Patients: Higher Temperature, Fibrosis, and Angiogenesis

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Background: Clinical trials have shown the effectiveness of systemic and local insulin therapy in improving wound healing. Diabetic wounds remain a challenge for healthcare providers. Impaired angiogenesis and reduced granulation tissue formation contribute to inadequate wound healing. The aim of this study was to investigate the effect of local insulin administration in acute and chronic diabetic wounds.

Methods: Eight diabetic patients presenting with full-thickness wounds, of different causes, were included in this study. Five wounds were due to necrobiosis, one to trauma, and two to postneoplasm resection. All wounds were treated with regular bedside treatment. In addition, half of the wound surface was treated with insulin and the other half did not receive insulin. Thermographic and biopsy specimens of the two sides were obtained on days 0 and 14. The presence of fibrosis, change in temperature, and amount of blood were evaluated.

Results: Significant differences in the number of vessels were observed on the insulin-treated side (96 ± 47) when compared with the no-insulin side (32.88 ± 45) ($p < 0.026$). The percentage of fibrosis (insulin: 44.42 ± 30.42 percent versus no insulin: 12.38 ± 36.17 percent; $p < 0.047$) and the mean temperature (insulin: 1.27 ± 1.12°C versus no-insulin: 0.13 ± 1.22°C; $p < 0.001$) were also significantly different between sides. No adverse events related to the study occurred.

Conclusion: The use of local insulin improves the formation of new blood vessels, increases fibrosis, and correlates with increased temperature. (*Plast. Reconstr. Surg.* 132: 1015e, 2013.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, II.

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THERAPEUTIC

Diabetes mellitus is one of the most common and best-known metabolic disorders[1] affecting the wound healing process.[1,2] Hyperglycemia reduces collagen deposits and delays wound remodeling.[3] Wounds of diabetic patients respond poorly to conventional treatment. This delay in wound healing has been associated with increased morbidity and mortality rates in this patient population.

The use of local injection of long-acting insulin-zinc suspension has been proven to accelerate wound healing without any major side effects.[4] Previous preclinical studies have successfully used intralesional insulin administration to restore collagen synthesis and formation of granulation tissue to normal values when administered during the early stages of the healing process.[5–11] In addition, decreased endothelial insulin growth factor-1 signaling has been implicated in impaired angiogenesis.[12] Moreover, studies in burned patients have shown that application of insulin into the burned wound improves wound matrix formation.[13] Other studies have reported increased angiogenesis and

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stimulation of fibroblast and keratinocyte proliferation following insulin treatment.[14–16]

In addition, research on burned patients has shown that application of insulin improves wound matrix formation.[13] Indeed, insulin increased angiogenesis and stimulated proliferation of fibroblasts and keratinocytes.[14–16]

Thermography has previously been used as a tool for assessing wound healing.[17] However, it has never been used to assess the changes in wounds of diabetic patients nor to correlate angiogenesis and fibrosis in response to the use of local insulin. The aim of this study was to investigate the effect of local insulin administration in acute and chronic diabetic wounds.

PATIENTS AND METHODS

Patients

This prospective and randomized study was performed on eight adult patients (six men and two women) with the diagnosis of diabetes mellitus type 2 who were selected from the general surgery wards at Hospital Central "Dr. Ignacio Morones Prieto," in San Luis Potosi, México. The mean patient age was 53 ± 33 years. All patients presented with a full-thickness wound of more than 25 cm^2. Five patients had wounds caused by necrobiosis, two had wounds caused by postneoplasm resection and one had wounds caused by trauma. Wound cultures with bacteria quantification were performed, and the study was not started until we had confirmation that there was no infection. For wounds caused by surgical tumor resection, the study started once it was confirmed that the patients were free of tumor. The average size of the wound was 261 ± 234 cm^2. The affected zones were located in the anterior thorax ($n = 1$, abscess of soft tissue of odontogenic origin), the back ($n = 2$, liposarcoma), the upper extremities ($n = 3$, two diabetic hands, one avulsion), the lower extremity ($n = 1$, diabetic foot), and the perineum (Fournier´s syndrome) (Table 1). This study was approved by the hospital's ethics committee (Reg. 70-11), and all patients provided informed consent.

Wounds were treated daily by physician. The wounds were divided into two parts of equal size; and a randomization table was used to select the side where insulin was going to be applied. After débridement of the wound, 10 units of neutral protamine Hagedon insulin was applied daily in the selected zone, which corresponds to 1-cm^2 middle region at a depth of 1 to 2 mm in the center of the area; insulin was not applied in the other part of the wound. Insulin was applied 1 hour after breakfast. Capillary blood glucose was measured 3 hours after the insulin was applied.

Tissue Biopsy

Biopsy specimens from the center of each side of the wound were obtained from the same place and in the same proportions at the beginning of the study and on day 14. A space of more than 2 cm was left between biopsy areas to avoid incorrect results due to spreading the effects of the insulin. Hematoxylin and eosin staining was used to study blood vessels, while percentage of fibrosis was visualized using Masson's trichrome stain. Image analysis was done with a 10× magnification of the 4-μm processed sections. An experienced pathologist blinded to the treatment reviewed the pathologic slides independently. The images were photographed with a digital camera (Olympus SP-320; Olympus America Inc., Allentown, Pa.) mounted on a microscope (Olympus CX31; Olympus) connected to a personal computer. Once the images were captured, the number of blood vessels in one randomly selected field was counted. Images were processed using the public domain software ImageJ v1.44 (National Institutes of Health, Bethesda, Md.). Fibrosis was evaluated by quantifying the percentage area of stain.

Thermography

Thermographic analysis was performed on the first day before the initiation of treatment and on day 14 to evaluate the overall wound temperature in comparison with an adjacent healthy area with equivalent temperature.[18] Infrared imaging was performed using a FLIR T400 infrared

Table 1. Demographic Information

Patient	Sex	Age	Area of the Body Affected	Cause of the Wound	Size of the Wound (cm^2)
1	Male	53	Anterior thorax	Necrobiosis	364
2	Male	30	Upper extremity	Necrobiosis	30
3	Male	40	Upper extremity	Trauma	140
4	Male	60	Upper extremity	Necrobiosis	175
5	Female	63	Back	Neoplasm resection	400
6	Male	62	Back	Neoplasm resection	750
7	Female	53	Lower limb	Necrobiosis	120
8	Male	63	Perineum	Necrobiosis	112

Table 2. Results of Statistical Analysis*

	Day 0			Day 14		
	Insulin	No Insulin	p	Insulin	No Insulin	p
No. of vessels	32.50 ± 28.39	70.37 ± 42.85	0.06	128.50 ± 40.76	103.25 ± 27.7	0.026
Fibrosis, %	23.70 ± 18.90	47.12 ± 30.50	0.09	68.12 ± 17.08	59.50 ± 17.42	0.047
Mean temperature difference with healthy skin (ΔT)	1.96 ± 1.25	2.70 ± 1.63	0.37	0.69 ± 0.46	2.83 ± 1.12	0.001

*Data are presented as mean ± SD. Wilcoxon/Kruskal-Wallis (rank sums) test was used for between-group comparisons.

camera (FLIR Systems, Wilsonville, Ore.) that had a 320 × 240 focal plane array of uncooled microbolometers with a spectral range of 7.5 to 13 lm and a thermal sensitivity of 50 mK at 30°C. The thermographic analysis was performed using FLIR QuickReport version 1.2 (FLIR Systems), which includes a tool to obtain maximum, minimum, and average temperature of a user-defined area. The assessors were blind to the study drug.

Statistical Analysis

Statistical analysis was carried out using JMP 7(1989-2007) (SAS Institute Inc., Cary, N.C.). Results are expressed as mean ± SD. Intraobserver and interobserver correlations were calculated with intraclass correlation coefficients (95 percent confidence intervals). A Wilcoxon/Kruskal-Wallis test (rank sums) (days 0 and 14) and Wilcoxon's signed rank test (difference between days 0 and 14) were used as appropriate, to determine the probability of significant difference. A value of $p \leq 0.05$ was considered to statistically significant.

RESULTS

Intraobserver correlation was determined by the same observer viewing 32 fields at two different times. The intraclass correlation coefficient of the number of vessels was $r^2 = 0.987$ (95% CI, 0.945 to 1) ($p < 0.0001$) and that of the percentage of fibrosis was $r^2 = 0.979$ (95% CI, 0.936 to 1) ($p < 0.0001$).

Interobserver correlation was determined by two observers viewing 32 fields at two different times. The intraclass correlation coefficient of the number of vessels was $r^2 = 0.9805$ (95% CI, 0.949 to 1) ($p < 0.0001$) and that of the percentage of fibrosis was $r^2 = 0.993$ (95% CI, 0.969 to 1) ($p < 0.0001$).

The result of the studied variables can be observed in Table 2. No significant differences in temperature patterns, number of blood vessels, or fibrosis were found at the beginning of the study; therefore, the samples were considered homogeneous. The difference in the number of blood vessels between 0 days and 14 days was 96 (± 47) in the side treated with insulin ($p < 0.001$) and 32.88 (± 45) in the side without insulin treatment ($p = 0.07$) (Fig. 1).

The difference in the percentage of fibrosis between 0 days and 14 days was 44.42 ± 30.42 percent on the insulin-treated side ($p < 0.01$) and 12.38 ± 36.17 percent on the non–insulin-treated side ($p = 0.46$) (Fig. 2).

When compared with the healthy skin, the average temperature difference, as detected by

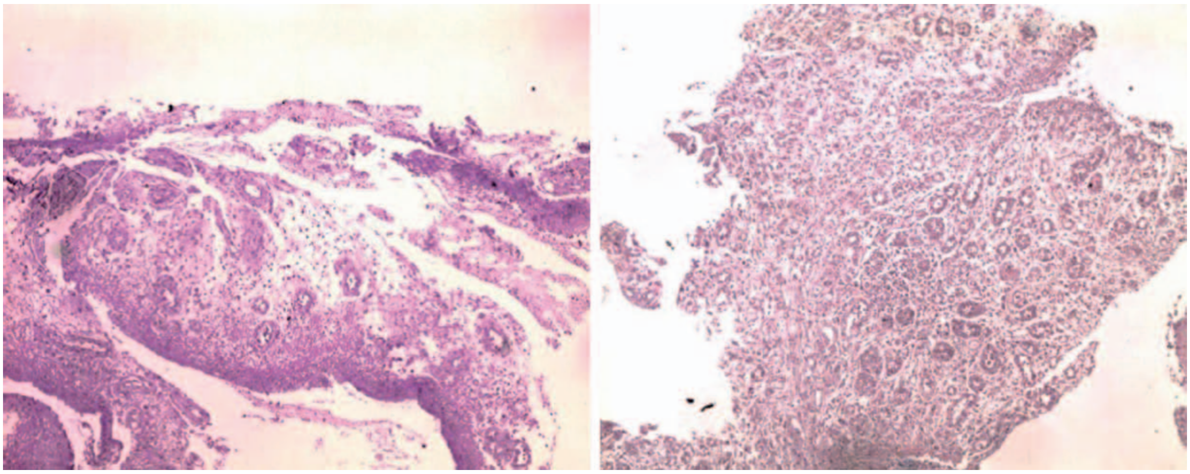

Fig. 1. Comparing the initial (*left*) and the final day (*right*) blood vessel counts.

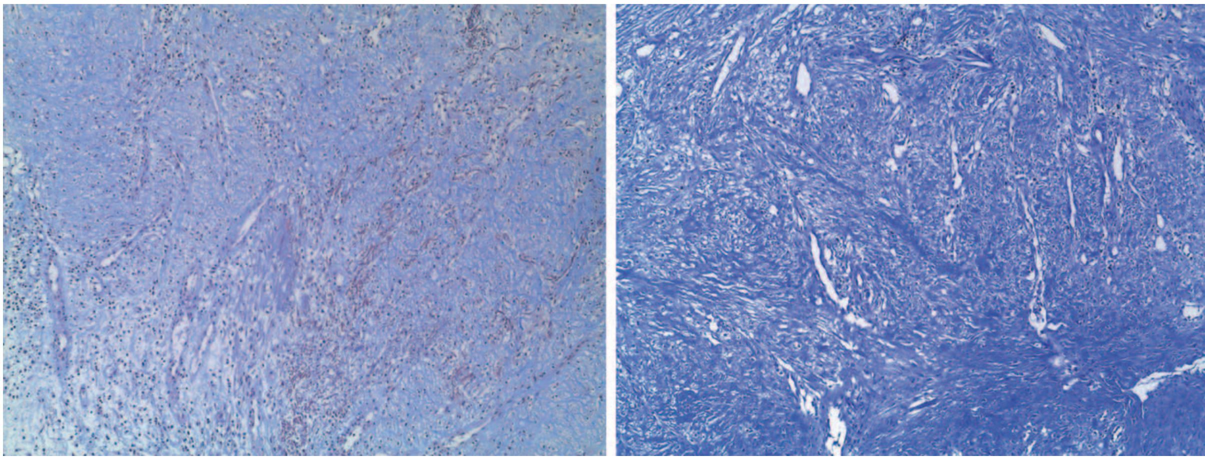

Fig. 2. Comparing the initial (*left*) and the final day (*right*) degree of fibrosis.

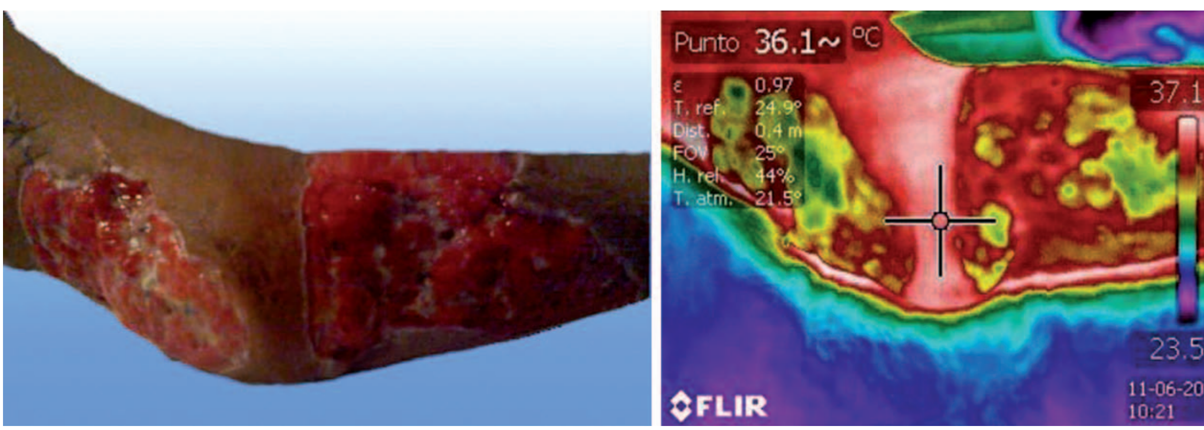

Fig. 3. Correlation of thermograph scan shows increased vascularity on day 14.

thermography, was 0.69 ± 0.46 on the insulin side and 2.83 ± 1.22 on the noninsulin side ($p < 0.001$) (Table 2). A statistical test (Wilcoxon's signed rank test) of one sample was performed based on the hypothesis that the value of the mean temperature should be the same on the first and last days. There was a significant difference on the insulin side (1.27 ± 1.12°C, $p = 0.001$) between days 0 and 14, but this difference was not significant on the noninsulin side (0.13 ± 0.22°C, $p = 0.76$). The above results indicate that thermographic analysis effectively detected temperature increases in wounds treated with insulin (Table 2 and Fig. 3).

None of the eight selected patients presented hypoglycemia or any other side effect.

DISCUSSION

Neovascularization is critical for successful wound healing.[19] Efforts have been made to induce new blood vessel formation in order to enhance tissue repair.[15,20–22]

Compared with previous studies,[11] in which insulin was used in the diabetic foot, we included diabetic patients with wounds in areas other than the diabetic foot, with five necrobiotic wounds, two wounds from resection of a neoplasia, and one wound secondary to trauma. In our study, insulin was administered subcutaneously in the wound at a depth of no more than 2 mm; therefore, we suggest that insulin absorption was higher in the wound tissue, with an unknown distribution into the systemic circulation, although this statement is still uncertain.[4]

Although several dose calculations have been made in experimental animals, limited literature is available on human subjects. We were not able to find studies in order to determine the most effective dose or the distance at which insulin acts effectively when it is applied locally. However, we noticed significant changes in vascularity distances of up to 2 cm. Our results suggest the possibility of local insulin application in zones of complex wounds (such as furrows, irregular areas, zones with difficult access, and so on) where it is required to form granulation tissue swiftly to improve healing and prevent complications.[14,15]

CONCLUSIONS

Our results show how the use of local insulin improves the formation of blood vessels and increases fibrosis, which correlates with change in temperature. Future studies that include a greater number of patients and that investigate the effect of insulin in diabetic patients in order to optimize dosing and treatment schedule are warranted.

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