A pilot trial using topical regular crystalline insulin vs. aqueous zinc solution for uncomplicated cutaneous wound healing: Impact on quality of life

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Manuscript received: April 8, 2013
Accepted in final form: September 16, 2013

DOI:10.1111/wrr.12122

ABSTRACT

When wounds are treated with regular insulin, they are also being treated with zinc; used in the formula to crystallize insulin molecules. It is not clear if regular insulin-accelerated wound healing is due to insulin, the zinc it contains, or both. Thus, we aimed to compare topical regular crystalline insulin (containing zinc) vs. aqueous zinc chloride solution to controls, on healing of open uncomplicated cutaneous wounds. In this randomized controlled pilot study, 90 nondiabetic patients were randomly assigned to one of three groups depending on the twice daily applications received; group I: regular insulin; group II: aqueous zinc chloride solution, and group III: 0.9% saline (control). A questionnaire was used to determine the effect of wounds on the quality of life. Both topical regular crystalline insulin (containing zinc) and aqueous zinc chloride solution enhanced healing of uncomplicated cutaneous wounds of nondiabetic patients, than control ($p < 0.001$), and hence improved patients' quality of life. However, regular insulin showed better results than aqueous zinc solution ($p = 0.015$), probably due to synergistic effect between insulin and zinc of its formulation. Healing rate was significantly higher in acute than chronic wounds ($p < 0.001$), in those ≤40 years than those >40 ($p = 0.004$), and in upper body wounds than lower body ($p = 0.015$).

Wound healing is a complex biological process influenced by several agents.[1] Many studies have showed positive effects of insulin on wound healing. In burn patients, insulin infusion was found to decrease mean donor-site healing times.[2] Another study on human volunteers found that wounds treated with topical insulin healed faster than saline-treated wounds.[3] In addition, Wilson et al. reported an improvement in healing of a case of resistant surgical wound with the use of insulin.[4] Moreover, insulin spray has been successfully used to treat diabetic ulcer patients.[5] However, no consensus about a single suitable method for routine clinical use of locally applied insulin has been reported so far.

Zinc is an essential mineral, involved in numerous aspects of cellular metabolism, immune function, protein synthesis, cell division, and wound healing.[6,7] The application of zinc oxide as paste, powder, or tape over the wound, thereby, was found to facilitate wound healing.[3]

Regular crystalline insulin (short acting) is available as a clear solution at neutral pH. Zinc in a concentration of 0.4% is added to allow the insulin molecules to self associate into hexamers.[8] Hence, when wounds are treated with insulin, they are therefore also being treated with zinc. If insulin accelerates wound healing, it is not clear if the increase in the rate of healing would be due to insulin (a known growth factor), the zinc it contains, or a combination of the two. The aim of this study was to evaluate the effect of using regular crystalline insulin (containing zinc) topically vs. zinc solution on healing of open cutaneous wounds, compared with controls.

MATERIALS AND METHODS

This randomized, placebo-controlled pilot study was conducted at Ain Shams University hospitals (Department of Dermatology, Burns and Plastic Surgery Department, Emergency rooms) through a period of 13 months. The study was conducted according to the Declaration of Helsinki Principles and was approved by the medical ethical committee of Ain Shams University. Ninety consecutive inpatients and outpatients with skin wounds were enrolled in the study, after signing an informed consent by the patients themselves or their guardians. Patients with acute wounds (burns or crush wounds) or chronic wounds (pressure ulcer) were eligible to participate. All patients were subjected to full medical history with emphasis on: cause, onset, course, and duration of wound(s), present extension or complication, history of smoking, previous and present medications that could affect the healing process (e.g. corticosteroids, oral antidiabetics, and wound treatment) and other relevant medications including: oral contraceptive pills and isotretinoin, relevant medical conditions (e.g. diabetes mellitus, atherosclerosis, history of abnormal scar formation, or history of chronic debilitating conditions). Serum zinc was measured for all patients before enrollment to exclude patients with abnormally low serum zinc level. Exclusion criteria included patients whose age >75 years, smokers, patients with immunosuppression, cardiovascular diseases, diabetes mellitus, any chronic debilitating disease, low serum zinc level, complicated wounds (e.g. bleeding or infection), history of abnormal scar formation, and/or previous or current medications likely to affect the outcome of the study.

Complete general and dermatological examination was done, as well as local evaluation of the wound with regard to: type; acute or chronic, distribution, shape, and area; and wound area was measured using a sterile transparent paper placed over the wound surface to mark wound borders. For the ellipsoid wound type, the largest diameter was measured using a ruler in mm, and the law $A = \pi r^2$ was used to obtain approximate wound area in mm^2. In addition, the depth of wounds was emphasized to be partial thickness.

Wounds were photographed at baseline to record the primary wound surface and shape using digital camera of type Cannon G7 Powershot 10 megapixels (Canon USA, Inc., Melville, NY). Sequential photographs was done using identical camera settings, lighting, and patient positioning, as baseline before treatment, on weekly basis until the end of follow-up period.

In cases of burns, burned skin was removed by an experienced surgeon before applying treatment. Before starting the therapeutic procedure, all wounds were fully washed with normal saline (0.9% NaCl). After eligibility confirmation, patients were randomly assigned to one of three groups (I, II, and III) and were treated as follows. Group I: 30 patients received 10 units (0.1 ml) of regular insulin "Humulin" (Eli Lilly and Company, Indianapolis, IN) in solution with 1 cc saline 0.9% for each 10 cm^2 of wound. Each 100 USP units (United States Pharmacopeial Convention, Inc.) of this type of insulin (regular) contain 10–40 mcg of zinc. Group II: 30 patients received sterile aqueous zinc chloride ($ZnCl_2$) solution with zinc concentration similar to that in regular insulin used in group I, which is around 20 mcg per 1 ml. The $ZnCl_2$ solution was obtained by dissolving 20 mg $ZnCl_2$ powder (obtained from Al-Gomhorya Company, Cairo, Egypt) in 1000 ml of sterile purified water, and the solution was sterilized in autoclave and kept in neutral glass container. The 0.1 ml of this solution was added to 1 cc normal saline (0.9% NaCl) and applied for each 10 cm^2 of the wound. Group III: 30 patients received sterile 0.9% saline (0.9% NaCl), twice daily as a placebo (control group). The topical treatment, assigned to be applied for each patient of the three groups, was used twice daily, left to dry for 30 minutes and covered with sterile cotton gauze. Blood glucose level was measured after 1 hour of the dressing on the first application. The dose was modified everyday depending on the wound area. The rate of wound healing was calculated as the difference between the primary and final wound area in (in mm^2) as a function of healing time (in days) and reported as mm^2/d. Wounds were considered fully healed when totally closed and epithelialized.

Assessment of quality of life by using Effect of Pain from the Wound on Quality of Life Questionnaire was done at the beginning and the end of the study. Questionnaire was modified according to Wound Symptoms Assessment Chart,[9] and Quality of life questionnaire for dialysis patients SF36 (the Short Form 36 Items Health Survey),[10] as well as Arabic translation of the RAND 36-item survey (developed from the Rand Corporation's health insurance experiment).[11] Details of the Questionnaire are available as Supplemental Information, published with the online version of the paper. The Questionnaire included 12 main questions, addressing seven specific areas of functional health status (physical functioning, role limitations due to physical health, role limitations due to emotional problems, wound status improvement, social functioning, pain, and general health). All questions were scored on a scale from 0 to 100, with 100 representing the highest level of functioning possible. Aggregate scores were compiled as a percentage of the total points' possible (step 1 chart). The scores from the questions that address a specific area of functional health status were then averaged together, for a final score within each of the dimensions measured (step 2 chart). All seven categories were scored in the same way at the beginning and at the end of the course of care to track the progress of them.

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS) version 17 (SPSS Inc., Chicago, IL). Comparisons between the three studied groups were done using the one-way analysis of variance followed by Tukey pair-wise comparisons. To study the differences between the three groups and factors influencing the healing rate, a two-way analysis of variance was used. The strength of association between duration of wound healing and wound area in the three study groups was calculated using the Pearson's correlation coefficient. To measure the strength of association between the rate of healing and duration of healing, after controlling for the effect of area, partial Pearson's correlation coefficient was computed. For comparisons between before and after scores for quality of life questionnaire Wilcoxon rank-sign test was used, and in the three groups, a two-way analysis of variance with repeated measures on one factor was done. The "change" of the seven parameters of the questionnaire in relation to other factors was compared using Kruskal–Wallis test. Spearman's correlation coefficient was used to measure the strength of association between the "change" of the seven parameters of the Questionnaire and the rate of wound healing. All p-values were two sided. p-Values <0.05 were considered significant, whereas p-values <0.001 were considered statistically highly significant.

RESULTS

Ninety consecutive patients (41 males and 49 females) with skin wounds were enrolled in this randomized placebo-controlled pilot study, divided into three equal groups, matched with regard to gender and age distribution, as well as wound area, types, and locations. One hour following applications, blood glucose level was still within normal range in the three studied groups' patients. Overall, comparing the rate of healing in the three groups revealed that group I showed the best rate of healing (mean ± standard deviation [SD] 53.5 ± 12.1), followed by group II (mean ± SD 46.4 ± 9.8), followed by group III (mean ± SD 36.1 ± 5.7), with a statistically significant difference ($p < 0.001$). Post hoc test revealed statistically higher healing rate in group I compared with groups II and III ($p = 0.015$ and $p < 0.001$, respectively), as well as in group II compared with group III ($p < 0.001$) (deleted statement about figures). Comparing male and female patients in each group revealed that the rate of healing did not show statistically significant difference ($p = 0.958$) (data not shown). Comparing those whose ages are ≤40 years and those >40 years in each group revealed that the rate of healing of wounds in those ≤40 years was statistically significantly higher than those >40 years ($p = 0.004$) (Table 1). Group I included 23 patients with acute wounds (20 patients

Table 1. Comparing the rate of healing of those ≤40 and >40 years of each group and among the three groups

	Age ≤40 years		Age >40 years	
Group	Number	Mean healing rate ± SD (mm²/day)	Number	Mean healing rate ± SD (mm²/day)
I	16	57.2 ± 11.3	14	49.3 ± 11.9
II	16	49.8 ± 9.2	14	42.7 ± 9.3
III	13	37.5 ± 6.1	17	35.1 ± 5.4

Two-way ANOVA results

Effect	*p*-value	Significance
Group	<0.001	Yes
Age	0.004	Yes
Group * type interaction	0.458	No

$p < 0.05$, significant; $p < 0.001$, highly significant.
SD, standard deviation.

with burns and three with crush injuries) vs. seven patients with chronic pressure sores. The mean rate of healing in this group was 59.8 mm^2/day for acute wounds (59.988 mm^2/day for burns and 58.724 mm^2/day for crush injuries) vs. 32.7 mm^2/day for pressure sores. Group II included 23 patients with acute wounds (18 patients with burns and five with crush injuries) vs. seven patients with chronic pressure sores. The mean rate of healing in this group was 51.5 mm^2/day for acute wounds (51.249 mm^2/day for burns and 52.462 mm^2/day for crush injuries) vs. 29.8 mm^2/day for pressure sores. Group III included 24 patients with acute wounds (21 patients with burns and three with crush injuries) vs. six patients with chronic pressure sores. The mean rate of healing in this group was 38.7 mm^2/day for acute wounds (36.848 mm^2/day for burns and 38.562 mm^2/day for crush injuries) vs. 25.9 mm^2/day for pressure sores. Comparing acute and chronic wounds in each group revealed that the rate of healing in acute wounds was significantly higher than in chronic wounds ($p < 0.001$) (Table 2). Comparing upper and lower body wounds in patients in each group revealed that wounds located on upper body showed higher rates of healing in the three groups ($p = 0.015$) (Table 3).

The duration of healing was directly correlated to the wound area in the three studied groups ($p < 0.001$). After controlling for the effect of area, there was excellent negative correlation between the rate of healing and duration of healing in days for all study groups ($p < 0.001$); i.e. the faster the rate was, the lesser the duration. Regarding quality of life questionnaire, comparing before and after scores, there was a significant difference between before and after for all questions in the three groups ($p < 0.001$ for all) (Table 4). Comparing the change of the score of each category of the seven

Table 2. Comparing acute and chronic wounds in patients of each group and each type of wound among the three groups regarding the rate of healing

	Acute wounds		Chronic wounds	
Group	Number	Mean healing rate ± SD (mm²/day)	Number	Mean healing rate ± SD (mm²/day)
I	23	59.8 ± 3.3	7	32.7 ± 2.1
II	23	51.5 ± 3.3	7	29.8 ± 2.1
III	24	38.7 ± 2.5	6	25.9 ± 1.8

Two-way ANOVA results

Effect	*p*-value	Significance
Group	<0.001	Yes
Type of wounds	<0.001	Yes
Group * type interaction	<0.001	Yes

$p < 0.001$, highly significant.
SD, standard deviation.

Table 3. Comparing lower body and upper body wounds in patients of each group and each location of wounds among the three groups regarding the rate of healing

	Lower body		Upper body	
Group	Number	Mean healing rate ± SD (mm²/day)	Number	Mean healing rate ± SD (mm²/day)
I	17	51.1 ± 14.1	13	56.5 ± 8.5
II	17	43.3 ± 10.4	13	50.5 ± 7.4
III	15	35.0 ± 6.4	15	37.2 ± 4.9

Two-way ANOVA results

Effect	*p*-value	Significance
Group	<0.001	Yes
Wound location	0.015	Yes
Group * location interaction	0.572	No

$p < 0.05$, significant; $p < 0.001$, highly significant.
SD, standard deviation.

parameters of the questionnaire among the three groups revealed that there was no statistically significant difference among the study groups ($p > 0.05$). In addition, comparing the improvement of the quality of life parameters in patients with acute wounds to those with chronic wounds showed no statistically significant difference, except for pain that showed better improvement in acute wounds ($p = 0.015$) (pain sensation diminished faster in the acute type than the chronic one). Improvement of all the studied parameters of the questionnaire was directly correlated with the rate of wound healing, particularly pain and general health improvement (Table 5).

DISCUSSION

Both regular insulin and topical zinc had been previously used to improve wound healing.[12,13] In general speaking, we found the use of either one on both acute and chronic uncomplicated cutaneous wounds to be safe and effective, as evidenced by enhanced wound healing compared with the control group. Our results regarding efficacy of local insulin in wound healing is matched with several other studies on both animals,[13–19] and humans,[3,4,20–22] with the most recent study by Xuelian et al. to treat full-thickness skin wounds made using a 5-mm diameter cornea punch on the dorsal skin of mice by

Table 4. Quality of life parameters before and after treatment in the studied groups

Parameter		Group I Mean ± SD	Group II Mean ± SD	Group III Mean ± SD	*p**
Physical nonfunctioning %	Before	51.2 ± 18.6	56.8 ± 22.6	46.7 ± 16.3	<0.001
	After	7.7 ± 11.1	8.3 ± 13.1	8.8 ± 12.0	
Role limitation due to physical health %	Before	65.0 ± 24.2	62.3 ± 21.3	54.2 ± 20.4	<0.001
	After	6.0 ± 8.0	6.2 ± 8.5	8.4 ± 12.5	
Role limitation due to emotional problems %	Before	65.8 ± 23.1	68.1 ± 18.1	64.2 ± 18.1	<0.001
	After	6.2 ± 6.5	6.8 ± 8.6	7.9 ± 9.7	
Wound status unimprovement %	Before	65.1 ± 20.1	67.5 ± 21.4	69.6 ± 20.4	<0.001
	After	6.7 ± 7.9	8.3 ± 10.0	7.9 ± 9.0	
Social nonfunctioning %	Before	56.7 ± 24.5	62.5 ± 27.7	53.3 ± 27.6	<0.001
	After	8.3 ± 12.0	5.8 ± 10.8	9.2 ± 13.9	
Pain %	Before	60.0 ± 20.0	60.6 ± 25.5	60.9 ± 23.5	<0.001
	After	10.0 ± 12.8	9.4 ± 12.0	15.4 ± 16.6	
Bad general health %	Before	77.9 ± 11.7	81.7 ± 12.2	76.3 ± 12.0	<0.001
	After	14.2 ± 15.3	13.3 ± 14.3	17.1 ± 14.5	

*$p < 0.001$, highly significant.
SD, standard deviation.

Table 5. Correlation between the improvement of the seven parameters of the Questionnaire and the rate of wound healing in the study groups

Improved parameter	Correlation with healing rate	Significance p (two-tailed)
Physical nonfunctioning	0.244	0.021*
Role limitation due to physical health	0.217	0.041*
Role limitation due to emotional problems	0.277	0.009*
Wound status unimprovement	0.302	0.004*
Social nonfunctioning	0.274	0.009*
Pain	0.446	<0.001**
Bad general health	0.439	<0.001**

*$p < 0.05$, significant; **$p < 0.001$, highly significant.

0.03-U insulin or saline. The healing time of wounds treated with insulin was significantly shorter than that of the saline treated wounds.[23] Though only minor changes were reported, yet, local insulin injection did affect blood glucose levels.[17] Hereby, it is worth mentioning that using topical insulin showed positive influence on cutaneous wound healing in our patients with no such side effect. In accordance, Rezvani et al. measured systemic glucose levels before and 1 hour after application of insulin topically. No patients developed signs or symptoms of hypoglycemia and glucose levels pre- and postapplication did not differ significantly.[22]

In contrast to our results, Gerber and Van Ort utilized a two-group, before-after design, where 29 geriatric subjects with decubitus ulcers were randomly assigned to either topical application of ten units of regular insulin (U.S.P.) twice daily, or saline, and found no significant differences. Thus, they suggested that insulin had no effect on accelerating wound healing.[20] The discrepancy between their results and ours could be due to different patients' selection, as they did not state whether the geriatric subjects had any other concomitant diseases that might impair wound healing as diabetes or atherosclerosis.

Concerning efficacy of topical zinc application in accelerating wound healing, our results are in accordance with previous animal,[3,12,24] and human studies' results,[3,18] in both zinc-deficient and zinc-sufficient candidates,[24] with reduced bacterial growth only in non-diabetic candidates.[12]

In the present study, we were the first controlled pilot study using insulin and zinc topically on human. The three study groups were matched with respect to type of wound (acute and chronic), location, and area, where initial wound sizes were similar and not significantly different. Comparing our groups, wound healing rates were significantly higher in group I (using topical insulin-zinc) than in group II (using only topical zinc solution with same concentration as that in insulin used by group I). Nevertheless, both trial groups showed significantly better healing rates than group III (using saline; control group). Greenway et al. compared the efficacy of insulin with that of a solution containing the same amount of zinc in accelerating the healing of standardized acute wounds in rats and humans. They concluded that topical insulin accelerates wound healing in humans and so does zinc.[3] Nevertheless, they did not clarify which of which showed better results. In another study, Zhang et al. found that the wound total DNA synthetic rate was increased with insulin-zinc administration than with zinc alone. In addition, the wound healing days were compared between two groups ($n = 7$ each) receiving local injection of either insulin-zinc or zinc alone.[17] In agreement with our results, the investigators found that the wound healing time was faster with insulin than with zinc.[18]

In our study, comparing patients with acute and chronic wounds in each group and among the three studied groups, it was found that the rate of healing showed statistically significant difference being higher in acute wounds, which matches the results stated by Rezvani et al. where, in their study, acute wounds healed faster chronic ones.[22] Comparing males and females healing rates among different groups and in each group in our study, we found that male and female patients showed statistically matched rates of healing, which is in accordance with Rezvani et al. results.[22] In contrast, Gerber and Van Ort stated that females healed significantly more slowly than males.[20] Nevertheless, no enough demographic data were available about their study. According to the age, we found the rate of healing of wounds in those ≤40 years to be statistically significantly higher than that of those >40 years, particularly in group II, in accordance with Rezvani et al.[22] Also comparing lower body and upper body wounds in our study showed statistically higher rate of healing of wounds in upper body, in contrast to Rezvani et al. results, as wound healing rates were not significantly different according to wound locations (upper and lower limbs).[22] Nevertheless, usually upper body lesions heal faster than lower body lesions due to several factors, among which stagnant circulation and pressure effects.

A positive correlation was found between the duration of healing in days and wound area in the three studied groups. After controlling for the effect of area, there was excellent negative correlation between the rate of healing and duration of healing in days for all study groups; i.e. the faster the rate was, the lesser the duration. These results are in accordance with other studies.[20,22]

Regarding the effect of wounds on the quality of life, comparing before and after scores, there was a significant improvement for all questions in the three groups, which means enhanced quality of life after healing. Moreover, improvement of all the studied parameters of the questionnaire was directly correlated with the rate of wound healing, particularly pain and general health improvement. In addition, improvement of all parameters was statistically matched among the studied groups, with no differences based on gender, age, wound location, and wound type, except for pain, which showed better improvement in acute wounds compared with chronic wounds, probably due to better healing rates, with faster reduction of pain in acute wounds' group.

Taken all together, the use of either topical crystalline regular insulin (containing zinc) or topical aqueous zinc on acute or chronic uncomplicated cutaneous wounds of nondiabetic patients seems to be safe and effective in enhancing acute and chronic wound healing and hence patients' quality of life. However, topical crystalline regular insulin could have better results, probably due to synergistic effect between the anabolic actions of insulin as a growth factor and the wound healing promoting influence of the zinc ions present in its formulation, for further studies on larger population of patients. Because of its long history of safe use in humans, crystalline regular insulin seems to be an effective wound healing agent without adverse effects. Therefore, we suggest that it can be used as an alternative to—or in combination with—currently used wound healing treatments. However, considering that the study did not control for the type of wound (both acute and chronic types were included) and given that stratification of data yielded small sample size in each subgroup, the consistency of our results needs additional studies. More accurate wound assessment methods, inclusion of diabetic patients, and proper investigation of the proposed synergism between insulin and zinc should be further considered in future studies.

ACKNOWLEDGMENTS

We would like to thank all members of Department of Dermatology, Burns and Plastic Surgery Department, and Emergency rooms, Ain Shams University Hospitals, for their support and cooperation. Extended gratefulness goes to Dr. Inas El Attar, Professor of Biostatistics, Department of Biostatistics & Cancer Epidemiology, National Cancer Institute, for sincere help with the statistical analysis of the study results.

Source of Funding: None.

Conflict of Interest: None.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Details of the Quality of Life Questionnaire and how to score the answers of the Questionnaire.