Zinc Topical and Iontophoresis Approaches in Treatment of Standardized Leg Burns: Animal Model

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ABSTRACT

This study was designed to compare the effects of Zinc application (Topical and Iontophoresis approaches) on burn wound healing of rats. It included 30 albino rats with second degree burn wound in their left hind limb and were divided randomly into two groups of equal number, 15 rats each, the first one received topical zinc and the other one received zinc iontophoresis. The parameters used for evaluation involved burn surface area measured by tracing wound perimeter and quantitative culture (number of bacterial growth) as an index of burn wound healing, pre and post treatment applications (after one and two weeks). These two parameters were measured 24 hours post-inflicted burn and 2 times post initiation of zinc therapy at one week interval and the same for colony count. The pre treatment results revealed no significant difference between the mean values of both parameters of both groups, while after two weeks of treatment applications, the findings showed that there was a significant difference between pre treatment and post two weeks for both groups with higher improvement in zinc iontophoresis group. Therefore, it may be of value for physical therapist to consider zinc iontophoresis as a method for treating burn patients.
Key Words: *Zinc application, iontophoresis burns, animal study.*

INTRODUCTION

Thermal injury primarily involves a destruction and disruption of normal anatomical and physiological integrity of the skin[14].
The ultimate goal of wound management is to allow to close as rapidly as possible, resemble the original tissue as much as possible, and produce the least amount of scaring[21].

Burn wound healing is a dynamic process involving physiological, biochemical and morphological alternations, so resembling the original tissue as much as possible and produce the least amount of scaring is the biggest goal in burn wound management[13].

Experimental variables can be better controlled in animal models which are to a great extent representative of the situation in humans[13].

Zinc is a component of numerous biological enzymes including those involved in digestion, metabolism, DNA and RNA synthesis, electron transport, and aerobic &anaerobic energy production[19].

Zinc is essential to good health and its benefits are (1) its action on the immune system, (2) wound healing accelerator, (3) it has extensive actions on sexual and sensory functions, and (4) it plays an important role in good healthy skin[12].

Zinc is more commonly used as zinc oxide in different topical preparations to treat skin lesions and dysfunctions[24].

Iontophoresis is the introduction of ions into the body for therapeutic purposes by means of a direct current through using the skin as a passage for administration of medication[15].

Many studies have reported numerous applications of iontophoresis for management of skin dysfunctions[11].

The main objective of this study is to evaluate the efficacy of zinc iontophoresis as a method of physical therapy comparing to the efficiency of topical zinc application on burn healing acceleration in rats.

MATERIAL AND METHODS

Samples (test animals)

The experiment of this study was carried out on 30 albino rats. Each rat was kept in a separate cage, in the animal house at the Physiology Department, Faculty of Medicine, Cairo University.

Their ages ranged from 6.00 to 7.20 months, with a mean value of 6.46 ± 0.49 months. Their body weight ranged from 165 to 210 grams, with a mean value of 180.76 ± 11.05 grams. All rats were free from any disease or injury that can affect the healing process and influence the results. All rats were burned on purpose by using stump to produce 2 cm^2 partial thickness burn on the left hind limb. Animals were classified into 2 groups of equal number.

Group I

Fifteen female albino burned rats served as topical zinc group and they were received topical zinc ointment. Their ages were ranging from 6 to 7.20 months, with a mean value of 6.466 + 0.520months, and their weights were ranging from 170 to 210 grams, with a mean value of 184 + 12.32 grams.

Group II

Fifteen female albino burned rats served as zinc iontophoresis group and they received zinc iontophoresis ointement. Their ages ranged from 6 to 7.15 months, with a mean value of 6.47 + 0.478 months, and their weights were ranging from 165 to 200 grams with a mean value of 177.53 + 8.89 grams.

Equipment Used

The equipment used were divided into 3 categories: tools for burn induction, in addition to therapeutic and measuring equipment.

Tools for induction of burn

- Hair removal cream (EVA).
- Alcohol: it is used to clean area from remnant of hair.
- Rectangular metal seal: with a 2 cm^2 contact surface area.
- Surgical forceps: it was used to pick up the seal after heating it.
- Surgical gloves: It was weared during the measurement and treatment phases.
- Thermometer: It was used to measure the temperature of the seal.
- Stopwatch: It was used to measure the time of contact between the seal and skin.
- Wooden plate: It was used to restrict animal movement during measurement and treatment phases.
- Sterile dressing (Tri-M Strip 6 x 8 cm post operative dressing) it was used to cover the wound except during measurement and treatment periods.
- Separate animal house.

Measuring tools

These tools were classified into two groups:

Tools for measuring wound surface area by tracing.

The following tools were used to conduct the wound surface area measurement:

- Sterilized transparency film.
- Tipped transparency marker.
- Carbon paper.
- Metric graph paper (1 mm^2).
- White paper.

Tools for taking swab.

MDIC swap made in Saudi Arabia.

Therapeutic Equipment

In this study, there were 2 forms of zinc application, iontophoresis device and zinc ointment.

Iontophoresis Device

It is a light weight iontophoresis device capable of providing an easy direct application to the skin, and manufactured by life tech., inc., model 6110 MP in Haustan, Texas, U.S.A. It can be supplied with either a battery or an adapter. Ionotophoresis unit is consisted of the following parts:

- DC Generators.
- Self-adhesive electrodes.
- Additional features of device.
  - Timer.
  - Lead clips.
  - An audible warning signal.

Zinc ointment

PROCEDURE OF THE STUDY

The procedures of the study were classified into the following various steps:

Animal handling and manipulation

Each rat should be grasped firmly around the shoulders, the fingers winding round abdomen. The animal should be turned onto its back and the tumb placed under its chin. In this position most rate relax, (Picking up phase).

The best method of restraint for the researcher and rat is that known as scruffing, i.e. making use of the loose folds of skin on the neck and back of the animal. After picking up process, the animal should be restrained in the left hand, thus leaving the right hand free to manipulate the procedure. The animal should first be picked up in the right hand, leaving the head and shoulders slightly protruding, then with the left hand the scruff should be taken between thumb and index finger. The rest of the fingers can then catch all the loose fur and skin down the back to the base of the tail in this position, the rat is not only immobilized but also very relaxed for most procedures[23].

Inducing burn injury procedure

The animal was anesthetized by placing the animal into closed cage in association with a piece of cotton with ether. The main aim of these procedures was to make the animal drowsy. The animal was strictly tied via four cotton threads to wooden plate prepared for this purpose. The hair at the upper part of the left hind limb was removed by hair removal cream and the remnant hair were removed by using a plastic stick, after that the skin was cleaned by a piece of cotton wetted with alcohol. The area of the skin prepared as above equal 3 cm^2 while the area intended to be burned equal 2 cm^2. The burns were inflicted by a rectangular metal seal with a 2 cm^2 contact surface area that was heated on a flame burner to 45°C and handled by surgical forceps then pressed immediately against the prepared skin segment for 20 seconds to produce partial thickness burns, according to Kaweski[16] and Cambier[6].

Measurement Phase

Measurement of Wound Surface Area (WSA) by tracing method

Measurement of WSA by tracing was performed 24 hours post burn, at 7^{th} day post burn and at 14^{th} day post burn. Each

measurement was done 3 times for the same wound and at the same time by the same investigator to establish reliability through obtaining the mean of these three times. The measurement of burn surface area was conducted by tracing the burn wound perimeter according to Bohannon and PFallor[4] and Kloth and Feedar[18] in the following steps: A sterilized transparency film was placed on the wound after cleaning it with antiseptic solution. The burn wound perimeter was traced with a fine tipped transparency marker. After that, the transparency film face, which faced the burn wound, was cleaned with antiseptic solution. The carbon paper was placed over the metric graph paper (1 mm^2). The traced transparency film was placed over the carbon paper with a white paper in between and transcribed the tracings onto the metric graph.

Assessment by taking swap from the wound (quantitative culture)

Three sterile rayon-tipped swab were obtained from each animal in both groups (treatment and control). The first swab was obtained 24 hours after burn infliction by carefully rubbing the swab across the wound surface. The second swab was obtained at 7^{th} day after burn infliction. The third swab was obtained at 14^{th} day after burn infliction. All swab were cultured on blood agar (aerobic and anaerobic) in both groups. The bacteriological medium used to culture all bacteria was blood agar in 100 mm petriplates (blood agar plates). Blood agar consists of triptic soy agar plus 5% sheep blood. The cultures were incubated at 37°C for 48 hours. The culture growth was made in a film and stained by using methyl violet stain, iodine solution and distilled water for the diagnostic identification of the type of various organisms. This previous method of doing culture was used by Cruichshank[10].

Treatment Phase

Topical application of zinc ointoment

In the first group, the burned area of each rat was cleaned firstly by antiseptic solution, the investigator placed by zinc ointment and was converted by sterile dressing which can be changed daily.

Application for iontophoresis technique of zinc

The basic principles of the application for iontophoresis technique of zinc achieving through these following steps.

Drug Concentration

The dosages and concentrations for medication administrated via iontophoresis are very important factor for principals application. Zinc oxide acts as a general antiseptic, may increase tissue healing for burn wound. The drug concentration of zinc oxide is 20% ointment from positive pole[7].

Skin Preparation

Preparing the skin by various rubbing with alcohol, this process decreases the chance of skin irritation by removing any oils or impurities. The skin should be shaved because hair is a form of contamination, the hair at the upper part of the left hind limb was removed by hair removal cream (veet)

Drug Placement

Medications contained in thicker creams (as zinc oxide) can be placed directly on the skin and then converted by a few thickness of gauze. The amount of drug applied varies according to each preparation and according to the size of the delivery electrode.

Electrode preparation and placement

The delivery electrode is placed over the desired site of application with care to maintain good contact between the skin and this electrode throughout the period of treatment. The return electrode is placed at a near by site on the same limb.

DC Generator (Connection and Current Application)

The current source is turned off and all dials were set to zero. The wire leads should be attached to the treatment electrodes. The leads are then connected to the DC generator. The selector switch was set to continuous galvanic current, current is then slowly increased to initial desired amplitude. Most treatment approaches of iontophoresis start with low amplitude (1-2 mA). The time of treatment was 10 minutes for each rat.

RESULTS

Results of Topical Zinc Group

Wound Surface Area (WSA)

As shown in table (1), the mean value of WSA in this group (pre-treatment application) was 2.046 ± 0.127 cm^2, while the mean values of WSA at 7^{th} day post burn, and at 14^{th} day post burn the topical control group were 1.719 ± 0.0734 cm^2 and 1.356 ± 0.105 cm^2 which represent 16.011% and 33.75% (the percentage of improvement) of the pre treatment application value respectively. As observed from table (1) in the topical zinc group, there were significant decrease in the WSA at 7^{th} and 14^{th} day post burn compared to those 24 hours post burn (pre-treatment application) (P = 0.00).

Colony Count

As shown in table (2), the mean value of colony count 24 hours post induced burn (pre-treatment) application was 263.47 ± 63.20 colony, while the mean values of colony count post one and two weeks (post treatment) were 42600 ± 7038.67 colony, 21340 ± 9279.67 colony which represent 16068,82% and 7999.5% of the pretreatment application (24 hours post-induced burn) respectively.

As observed from table (2) in the topical zinc (control group), there were a significant increase in colony count at 7^{th} and 14^{th} day (post-treatment) compared to those 24 hours post induced burn (pre-treatment) values. (P = 0.00).

Results of Zinc iontophoresis group

Wound Surface Area (WSA)

As shown in table (3), the mean value of WSA in this group (second group) (pre-treatment application was 2.0133 ± 0.02160 cm^2, while the mean values of WSA at 7^{th} and 14^{th} days post burn in the treatment group (iontophoresis group) were 1.1193 ± 0.07314 cm^2 and 0.31 ± 0.3205 cm^2 which represent 44.40% and 84.60% of the pre-treatment application value respectively. As observed from table (3) in iontophoresis group, there were significant reduction in the WSA at 7^{th} and 14^{th} days post burn compared to those pre treatment values (P = 0.00).

Colony Count

As shown in table (4), the mean value of colony count 24 hours post induced burn (pre-treatment application) was 275.67 ± 41.66 colony, while the mean values of colony count post one and two week of treatment applications were 36400 ± 5315.74 colony, and 15000 ± 9068.71 colony which represent 13104.19 % and 5341.29% of the pre treatment application (24 hours post-induced burn) respectively. As reflected from table (4) in the iontophoresis group (second group) there were significant increase in colony count after 7^{th} and 14^{th} days of treatment application compared to those pre-treatment value (P = 0.00).

Table (1): Mean, S.D. and Percentage of Improvement of WSA at the 7th and 14th days post treatment application in topical zinc group (first group).

Statistic	WSA (cm^2)					
	Pre*	Post (1)	Pre	Post (2)	Post (1)	Post (2)
Mean	2.046	1.719	2.047	1.356	1.719	1.356
SD±	0.127	0.0734	0.127	0.105	0.0734	0.105
Difference	+0.3277		+0.6907		+0.363	
t-value	+7.275		+15.979		+12.624	
P-value	0.000		0.000		0.000	
Level of sign.	S		S		S	
% of improvement	16.011%		33.75%		21.12%	

Pre*: 24 hours post burn.
Post (1): at 7th day post burn.
Post (2): at 14th day post burn.

Table (2): Mean, S.D., and Percentage of Improvement of Colony Count at the 7th and 14th days post treatment application in Topical Zinc Group (First Group).

Statistic	Colony Count					
	Pre*	Post (1)	Pre	Post (2)	Post (1)	Post (2)
Mean	263.47	42600	263.47	21340	42600	21340
SD±	63.20	7038.67	16.32	9279.76	8038.67	9279.76
Difference	-42336.53		-21076.53		+21260	
t-value	-23.293		-8.788		+7.048	
P-value	0.000		0.000		0.000	
Level of sign.	S		S		S	
% of improvement	16068.82%		7999.6%		49.91%	

Pre*: 24 hours post burn.
Post (1): at 7th day post burn.
Post (2): at 14th day post burn.

Table (3): Mean, S.D., and Percentage of improvements of WSA at the 7th and 14th days post treatment application in zinc iontophoresis group (second group).

Statistic	WSA (cm^2)					
	Pre*	Post (1)	Pre	Post (2)	Post (1)	Post (2)
Mean	2.013	1.119	2.013	0.310	1.119	0.130
SD±	0.022	0.073	0.022	0.321	0.073	0.321
Difference	+0.894		+1.7033		+0.8093	
t-value	+49.843		+20.658		+9.132	
P-value	0.000		0.000		0.000	
Level of sign.	S		S		S	
% of improvement	44.40%		84.60%		40.20%	

Pre*: 24 hours post burn.
Post (1): at 7th day post burn.
Post (2): at 14th day post burn.

Table (4): Mean, S.D., Percentage of improvement of colony count at the 7th and 14th days post treatment application in zinc iontophoresis group (Second Group).

Statistic	Colony Count					
	Pre*	Post (1)	Pre	Post (2)	Post (1)	Post (2)
Mean	275.67	36400	275.67	15000	36400	15000
SD±	41.66	5315.74	41.66	9068.71	5315.74	9068.71
Difference	-36124.33		-14724.33		+21400	
t-value	-26.371		-6.289		9.81	
P-value	0.000		0.000		0.000	
Level of sign.	S		S		S	
% of improvement	13104.19%		5341.29%		58.79%	

Pre*: 24 hours post burn.
Post (1): at 7th day post burn.
Post (2): at 14th day post burn.

DISCUSSION

The findings of the results of this control randomized study, concerning the comparison between two various approaches of Zinc therapy (topical zinc and zinc iontophoresis) for promoting rate of healing on second degree burn in rats, revealed a significant reduction in the burn surface area and colony count after application of zinc iontophresis for a period of one or two weeks compared with topical zinc approach, i.e. the rate of healing in zinc iontophresis group was faster than that of topical zinc group.

These findings regarding the significant decrease in burn surface area at the end of the first and second weeks post initiation of zinc iontophoresis confirm the observations and outcomes of other studies which reported by Murray[20], Wilkinson and Hawke[24], Cakman[5], Kirschmann[17], Costella and Jesk[9], Tarnow[22], Agren[1], Balogun[3], Agren[2], and Cornwall[8].

The chronology of wound healing can be divided into two main phases: first removal of dead tissue, i.e. wound cleansing and secondly, actual healing by which new tissue is formed.

In poorly healing wounds such as deep burns, the breakdown processes must be further stimulated to achieve repair and healing.

There are many scientific researches and studies that support the importance of the zinc and its applications as a wound healing accelerator:

Zinc probably improves the anabolic phase of healing (reformation of new tissues) by providing the wound sufficient energy metabolism that required for repairing process, therefore zinc acts as a vital role in promoting epithelization and enhancing reepithelization process during wound healing of partial thickness of burns, Agren[1] and Agren[2].

Since wound healing is a multiphase, complex phase, the individual phases may be influenced by single factors or multi factorial approach can be taken to cover the whole healing process more fully.

The topical zinc or zinc iontophoresis represents such a multi factorial approach to stimulate normal repair and accelerate wound healing by decrease the rate of inflammation and bacterial growth, i.e. zinc acts as antibacterial effects Balogum[3]. Furthermore, zinc is essential for the proper functioning of the immune system and body natural defense against infection and viral compounds Cakman[5].

The components of zinc (numerous biological enzymes, forms of protein, essential trace elements or minerals, and electrolytes

[17] and Murray[20], evidently favourably influence both the catabolism (breaking down and removing dead tissues) as well as the anabolism phase (repairing and reforming of the new tissues) of wound healing.

Cornwall[8] proved that zinc iontophoresis plays an important role in increase collagen secretion which facilitate wound healing in forms of gradual and continual increase in the wound strength and improve wound vascularity.

Costella and Jesk[9] found that zinc iontophoresis increases the modification process of electrophysiological properaties of damaged or injured tissues.

Tarnow[22] showed that zinc oxide as topical form of treatment activates endogenous growth factor which plays an important role in the facilitation of keratinocyte to migrate and the keratinzation process improves the activity of epidermal cell in proliferate stage.

Wilkinson and Hawke[22] recommended the oral application of zinc leads to increase tensile strength and tissue elasticity in chronic leg ulcer.

The differences in the reduction of burn surface area between topical zinc and zinc iontophoresis groups may be due to the action of iontophoresis for drug delivery or facilitate diffusion of zinc into the skin, and this process is considered as a useful index to enhance trandermal penetration of the drug while providing the therapeutic effects of iontophoresis therapy.

From second part of the study, it concluded that zinc iontophoresis is effective method in reducing the colony count after one and two weeks of treatment application. The above results can be explained based on the therapeutic efficiency of zinc iontophoresis to increase the function of immune cells[20] as well as its bacterial effects[1,2] in addition to acceleration of the inflammatory phase of burn healing[1,2].

From the previous discussion of the current findings and according to related research studies in the field, it can be suggested that there is a clear reduction of burn surface area and decrease in colony count of bacteria after application of zinc iontophresis therapy as an indicator for acceleration of burn healing.

CONCLUSION

It can be concluded that both topical zinc and in particular zinc iontophoresis appear to be effective and safe methods of the treatment to enhance rate of burn wound healing. Measurement of WSA by tracing and type of bacteria developed (colony count) have been used to clarify the positive effects of two approaches of zinc applications (topical and iontophoresis) on bacterial growth and acceleration rate of wound healing.

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الملخص العربي

استخدام الانتقال الموضعي والانتقال التأيني للزنك في علاج حروق الساق : نموذج حيواني

الهدف من البحث : كان الهدف من هذه الدراسة قياس تأثير الانتقال الأيوني لمرهم الزنك عن طريق استخدام التيار الكهربائي المباشر على التئام جروح الحروق في الفئران لتحديد أفضل الوسائل العلاجية التي تساعد على سرعة التئام جروح الحروق .

مواد وأساليب البحث : أجريت هذه الدراسة على أربعين فأرا (ألبينو) وذلك في بيت الحيوانات التابع لقسم الفسيولوجي بكلية الطب جامعة القاهرة ، وممن تعرضوا لحرق من الدرجة الثانية مساحته ٢×٢ سم٢ في الطرف السفلي الأيسر لكل فأر وذلك عن طريق تسخين قطعة معدنية مستطيلة الشكل إلى درجة ٤٥ درجة مئوية ووضعها على المكان المارد حرقه لمدة ١٠ ثواني وقد تم تقسيم حيوانات التجربة عشوائيا إلى مجموعتين .

المجموعة الأولى : تتكون هذه المجموعة من عشرين فأرا ممن يتلقون مرهم الزنك موضعيا على الحرق وذلك لمدة خمس دقائق ثلاثة جلسات في الأسبوع ولمدة أسبوعين (١٤ يوم) وذلك بعد أن يتم تطهير الجرح تطهيرا كاملا .

المجموعة الثانية : تتكون هذه المجموعة من عشرين فأرا أيضا ممن يتلقون مرهم الزنك عن طريق الانتقال الأيوني من خلال جهاز التيار الكهربي المباشر على الحرق .

وقد تم قياس سرعة التئام الحروق بطريقتين : قياس مساحة السطح المحترق أو عمل مزرعة بكتيرية لتحديد نوع البكتيريا الموجودة بالحرق كمقياس لالتئام الحرق ، لقد تم عمل القياسات بعد الحرق مباشرة بأربع وعشرين ساعة ثم بعد أسبوع واحد من العلاج ثم بعد أسبوعين من العلاج أيضا .

لقد تم عمل الجداول الإحصائية اللازمة لإجراء المقارنة بين المساحة الفعلية للجزء المحترق وعدد البكتيريا النامية قبل وبعد علاج الحرق بالزنك الموضعي أو الزنك المتأين (بين المجموعتين) .

نتائج البحث : قد أظهرت نتائج البحث وجود فروق ذات دلالة إحصائية بالنسبة للمجموعة الثانية (التي حصلت على مرهم الزنك من خلال التأين الكهربائي) بعد فترة التجربة بمقارنتها بالنتائج قبل بدء التجربة . كما أظهرت أيضا وجود فروق ذات دلالة إحصائية بالنسبة للمجموعة الأولى (التي حصلت على مرهم الزنك موضعيا على الجرح) بمقارنتها بالنتائج قبل بدء التجربة . وبمقارنة نتائج المجموعة الثانية بالمجموعة الأولى وجد أن هناك تحسن واضح وملحوظ في المجموعة الثانية عنه في المجموعة الأولى بسبب انتقال هذا المركب (الزنك) عن طريق التأين الكهربي (تأين مزدوج) . مما يعني أن استخدام مرهم الزنك عن طريق الانتقال التأيني باستخدام التيار الكهربي المباشر يساعد على التئام الحروق أكثر من التئام الحروق عن طريق استخدام الزنك موضعيا والتي تعطي أفضل النتائج وأسرع زمن للالتئام مما يؤدي إلى سرعة تمام لاستشفاء .

وبعد مناقشة النتائج وجد أن انتقال مركب الزنك عن طريق التأمين الكهربي يعتبر من الوسائل ذات الفاعلية في التئام الحروق .