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Enhancement of Re-epithelialization with Topical Zinc Oxide in Porcine Partial-Thickness Wounds

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We investigated the effect of locally applied zinc on the healing of partial-thickness skin wounds in the domestic pig using two zinc compounds (zinc oxide and zinc sulfate) in two different vehicles (a gauze compress and a collagen sponge). The rate of re-epithelialization was determined morphometrically 48 and 64 hr after infliction of standardized square wounds (4.8 cm² and 400-μm deep) with an electrokeratome. Zinc oxide in gauze significantly ($P < 0.05$) increased re-epithelialization of the wounds (33% more epithelialized than control wounds after 64 hr) and in collagen sponge (76% more epithelialized than control wounds after 64 hr). Zinc sulfate had no such stimulatory effect at any dosage or vehicle used. Our results show that topical zinc oxide enhances re-epithelialization of partial-thickness wounds in nutritionally balanced pigs and that the mode of delivery of zinc is probably critical for achieving the beneficial healing effect of zinc. © 1991 Academic Press, Inc.

INTRODUCTION

Zinc is an essential trace element of which about 2 g is found in the adult human body. At least 200 enzymes in different biological systems are dependent on the presence of the zinc ion. Among these zinc-dependent enzymes, DNA and RNA polymerases are crucial during tissue repair as they affect cell proliferation and protein synthesis.

In accordance with the biochemical role of zinc a reduced synthesis of DNA, reduced deposition of granulation tissue, decreased tensile strengths in skin incisions, and delayed closure rates in excised wounds in zinc-deficient rats have been demonstrated [1, 2]. Zinc supplementation restored to normal the tensile strengths of the incisional and healing rates of the excisional wounds [2]. It has been clinically shown that the healing of leg ulcers is delayed in patients with subnormal serum-zinc levels [3]. Zinc given as oral and topical zinc sulfate or as topical zinc oxide normalizes impaired healing ability in these patients [3–5].

Numerous attempts to augment either primary or secondary wound repair in subjects with normal zinc status have so far, however, been inconclusive when either oral [3], parenteral [6, 7], or topical [8–10] zinc administration has been tried. *In vitro,* however, Lally *et al.* [11] have shown that zinc oxide increases the outgrowth of porcine 300-μm skin explants and *in vivo,* Hallmans and Lasek [12] reported a transient beneficial effect of topical zinc oxide on the size reduction of open wounds in both zinc-deficient and zinc-nondeficient rats. We therefore considered it worthwhile to study the effect of exogenously applied zinc on wound healing in well-nourished domestic pigs. For this purpose, we used our recently standardized and validated wound model in assessing re-epithelialization [13].

MATERIALS AND METHODS

Principles for Use of Animals and *The Guide for Care and Use of Laboratory Animals* (National Institute of Health) were followed. Four Yorkshire female piglets weighing between 18 and 24 kg were used. The piglets were provided with tap water and a basal swine pelleted diet (Arizona Feed, Eagle Milling Co., Tucson, AZ) containing 52 mg zinc (as zinc oxide)/kg. The animals were kept in individual cages.

Surgery. The animals were placed under general anesthesia with halothane. The dorsal skin was shaved with electric clippers, and cleansed with 70% ethanol, chlorhexidine gluconate (4% wt/vol), and sterile saline (0.9% NaCl wt/vol). A total of 16 or 24, 2.2 × 2.2-cm partial-thickness wounds were made on each pig by the same individual with an electrokeratome (Storz Instrument Co., St. Louis, MO) set to a depth of 400 μm. This procedure removes the entire epidermis with hair follicles, sebaceous and sweat glands remaining in the wound

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TABLE 1

Zinc Contents of the Dressings and the Effect of Zinc in Gauze and in Collagen Sponge on Re-epithelialization

		Ef		al wound area	
Gauze					
+PVP[c]					
+$ZnSO_4$ + PVF		5.8		54.8	.0 (21)
+$ZnSO_4$ + PVF		3.2	.6 (	66.6	.8 (28)
+$ZnSO_4$ + PVF		2.1		18.0	.4 (32)
+ZnO + PVP			.4 (1	84.8	.6 (31)
Collagen sponge					
+PVP					
+$ZnSO_4$ + PVF					ND
+ZnO + PVP	10	41.8			

[a] Determined in triplicates and given as the total available *eleme*
[b] $X \pm$ SEM.
[c] Contained 5 mg PVP/g as determined gravimetrically.
[d] Number of sections assessed.
[e] ND, not determined.
[*] $P < 0.05$ compared to respective control treatment (gauze + P' llagen s' 'VP, respec at each

bed as the source of re-epithelialization apart from the wound edges [13]. The wounds were inflicted in groups of two wounds with 1 cm of intact skin between the two wounds and the groups were placed more than 3 cm apart. The area of the wounds corresponded to 1.5% of the total body surface area.

Wound treatments. Two different vehicles for the zinc compounds were used: an absorbent gauze composed of 100% cotton (USP XXI type IV) and a glutaraldehyde cross-linked bovine collagen sponge (C. Freudenberg, Germany). The absorbency of the gauze dressing was 0.1 ml saline/cm^2 dressing material and of the collagen sponge 0.16 ml/cm^2. A single layer gauze was impregnated with a suspension of zinc oxide (25 g ZnO/liter), with three solutions of zinc sulfate (2, 20, and 200 g $ZnSO_4 \cdot 7H_2O$/liter, respectively) all containing 2.5 g PVP (polyvinyl pyrrolidon $M_r = 90{,}000$)/liter, or by 2.5 g PVP/liter alone, then dried in an oven and folded 8 times to dressings as described elsewhere [14]. The collagen sponge was impregnated manually with 5 g ZnO/liter plus 0.5 g PVP/liter, 3.5 g $ZnSO_4 \cdot 7H_2O$ plus 0.5 g PVP/liter or 0.5 g PVP/liter alone and lyophilized. All dressings were sterilized with ethylene dioxide. The measured zinc content of the dressings is shown in Table 1.

Assignment of the different dressings to each group of two wounds was made in such a fashion to avoid systematic influences of the anatomic location and adjacent dressings. Dressings were applied topically onto the wounds as single doses after a short period of hemostasis using gauze swabs. All dressings were first moistened with 0.1 ml/cm^2 sterile saline, then covered with a semipermeable adhesive polyurethane membrane (Tegaderm, 3M, St. Paul, MN) to prevent dehydration and damage to the wounds. An elastic jacket was finally fitted over the dressings and around the pigs.

Healing assessment. The dressings were not changed until harvesting 48 or 64 hr postoperatively. Wounds were inspected and photographed, and the dressings were then removed in cases of nonadherence, otherwise left on the wounds. The wounds were obtained for histological evaluation by a wide excision to the muscular layer, fixed in buffered 4% formaldehyde and embedded in paraffin. Eight sections from each paraffin block were selected through a random number generator and stained with hematoxylin-eosin. For each section the percent of the wound surface length covered with at least one epithelial cell layer was measured at a total magnification of 63× [13]. Histological sections where the epithelium was evidently torn off were disregarded. For example, the half of one wound which was covered with gauze after histological processing was significantly more epithelialized than the half of the same wound which was not covered with gauze; 63.6 ± 4.6 versus 38.3 ± 6.0%. The influence of the different treatments on the dermal inflammatory response was also assessed. The degree of inflammation was judged as slight, moderate, or severe according to the density of inflammatory cells and the depth of inflammation was rated as superficial, halfway, or complete. All histological examinations were performed without prior knowledge of which treatment group the sections belonged to.

Zinc determinations in serum were carried out after the surgical trauma, due to the association of trauma and depression of the serum-zinc level [15]. Blood samples were obtained from one of the ear veins under halothane anesthesia after wounding and at the end of the treatment period after excision of the wounds for histology.

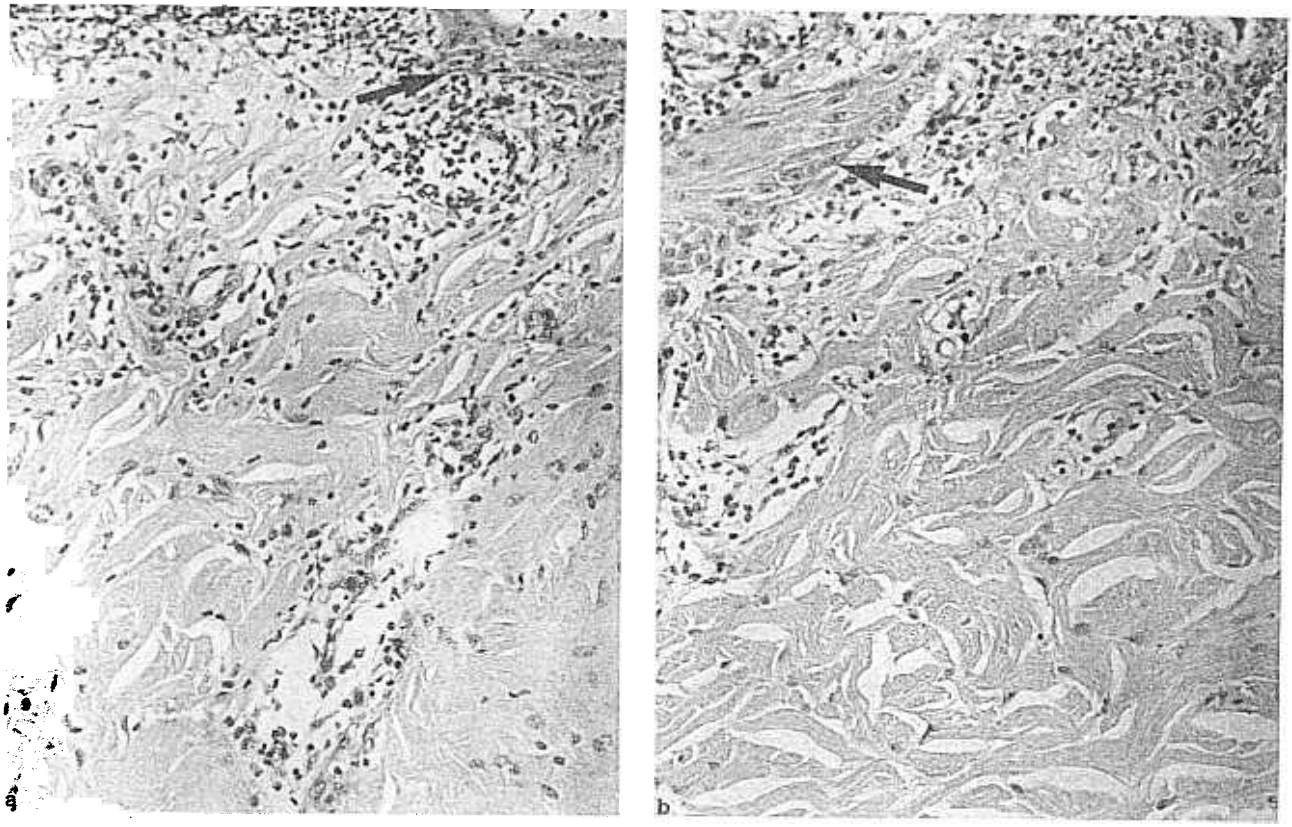

FIG. 1. The appearance of the upper dermis in wounds treated for 48 hr with gauze + (a) PVP; (b) zinc sulfate (65 μg zinc/cm²) + PVP; and (c) zinc sulfate (620 μg zinc/cm²) + PVP. Arrows indicate the edge of epithelium resurfacing the wounds. Note less inflammation in zinc sulfate treated wound (b) compared with control treated wound (a). A more pronounced inflammatory reaction was, however, seen with the highest zinc sulfate dosage and some parts of these wounds showed a very dense infiltrate of leukocytes (c). Hematoxylin-eosin, 215×.

The serum was diluted 10 times with double deionized water (Milli-Q, Millipore) before analysis. Dressings and diet were wet-ashed in 6 *N* nitric acid at 130°C for 30 min. Zinc concentrations in the solutions were determined using flame atomic absorption spectrometry (Perkin Elmer 305A and 1100B, Norwalk, CT).

Statistics. The mean (X) and standard error (SEM) for each treatment group were computed based on all individual section percent values. Duncan's multiple range statistics were calculated by computer to evaluate the statistical significance between the treatment groups. Differences in the serum-zinc level before and after treatment were determined using the paired t test. Differences were considered significant if $P < 0.05$.

RESULTS

At removal all dressings were moist. Most of the dressings adhered to the wounds after 48 hr but after 64 hr few dressings adhered. Macroscopically, wounds treated with the highest zinc sulfate dosage were much paler than the other wounds. The degree of epithelialization could not be estimated by the naked eye.

Re-epithelialization was not affected by PVP alone, neither when the gauze compress nor when the collagen sponge were applied (data not shown). Wounds treated with the non-zinc-medicated collagen sponge were less epithelialized than the wounds treated with non-zinc-medicated gauze. When zinc oxide was applied, the epithelial wound coverage was significantly higher compared with controls, irrespective of which vehicle was used (Table 1). No such beneficial effect on re-epithelialization was found with zinc sulfate in either vehicle or dosage. On the contrary, epithelialization was significantly inhibited at the highest zinc sulfate dosage (Table 1).

In order to explain the retarded epithelialization of wounds treated with collagen sponge the cytotoxicity of sponge and gauze extracts was studied in cultured 3T3 fibroblasts [16]. The incorporation of [^{3}H]-thymidine in the cells treated with the collagen sponge extract was significantly decreased compared with the gauze extract (data not shown), results which suggest that the collagen sponge released toxic substance/substances.

The infiltration of inflammatory cells was mostly confined to the perivascular and periadnexal areas, and only in some groups with moderate inflammation was the infiltration diffuse between collagen bundles. Control wounds treated with either gauze or collagen sponge but without zinc added showed a slight inflammation ex-

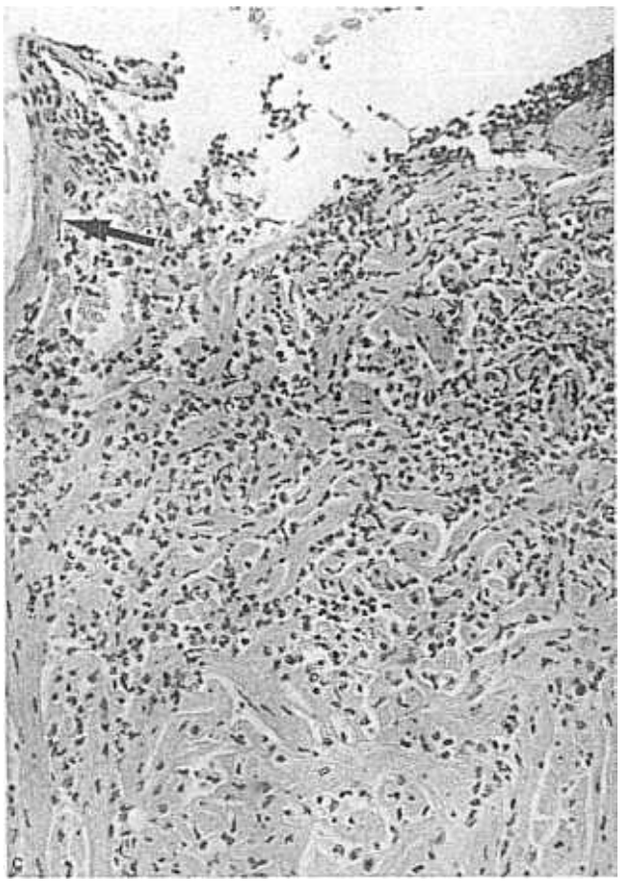

FIG. 1—*Continued*

tending halfway down in the dermis. When zinc was applied, however, the inflammatory reaction was less pronounced, except when the highest dosage of zinc sulfate was used. The latter treatment caused a moderate, diffuse inflammatory reaction involving the complete depth of the dermis (Fig. 1). Between the two time points 48 and 64 hr, differences in dermal inflammation were seen only in the highest zinc sulfate dosage group where a denser infiltration of polymorphonuclear leukocytes in the upper dermis was found at 64 hr. No foreign-body reaction was observed in the tissue of any group.

The serum-zinc level increased significantly during the treatment period; the pretreatment value being 0.70 ± 0.05 μg/ml (X ± SEM) whereas the post-treatment value was 0.83 ± 0.06 μg/ml.

DISCUSSION

Zinc oxide used topically on wounds is a folk remedy believed to promote healing. However, there is to date no clear scientific proof as to its effectiveness on wound healing in humans or experimental animals which are not zinc deficient [2, 8–10]. In this study we investigated the effect of two different zinc compounds—zinc oxide and zinc sulfate—on one aspect of wound healing, namely, re-epithelialization. The results show that topical zinc oxide treatment enhanced the re-epithelialization of partial-thickness skin wounds in normal pigs but topical zinc sulfate had no effect. The highest zinc sulfate dosage inhibited epithelial healing probably due to local toxic effects as reported earlier for wounds on rats [17].

The wounds treated with the highest zinc sulfate dosage had a paler clinical appearance than the other wounds, which could possibly be due to an edema, as a pronounced inflammatory response was seen histologically in the dermis with this zinc dosage. However, with the lower zinc sulfate dosages and with zinc oxide the dermal inflammation decreased compared with controls. This anti-inflammatory activity of zinc, which has been reported earlier [18], and the increased serum-zinc level indicate that zinc penetrated into deeper tissues and was absorbed into the general circulation. However, we did not include a parallel control group for the serum zinc measurements. This should ideally comprise pigs with the same wounds treated only with the non-zinc-medicated vehicles. Absorption of zinc from wounds treated topically with zinc oxide [10, 12] or zinc sulfate [17] has been shown in rats.

The discrepancy between our results with topical zinc oxide and those of other investigators who found that topical zinc oxide was ineffective on tensile strength, wound contraction, and time to complete healing of nondeficient skin wounds [8–10] might be ascribed to factors such as type of vehicles, dosages, and differences in healing characteristics between species. For this study, we chose the pig primarily because pig skin is similar to that of humans. In addition, the above mentioned investigations involved large full-thickness wounds on rodents which heal mainly by contraction and to a lesser extent by re-epithelialization.

Although it has been found that zinc is proliferative for epidermal cells from zinc-deficient rats [19], the mechanisms by which the supplemental zinc exerts its beneficial effect on epithelial healing are unclear. The course of healing of partial-thickness porcine wounds has been studied extensively by Winter [20]. He found that epidermal regeneration was achieved by cells from wound margins and skin adnexae. Within the first 24 postoperative hours the main mechanism of wound resurfacing was migration by epidermal cells. The mitotic activity appeared to be maximal between 48 and 72 hr. Therefore, it is possible that the rapid cell division in wounds is connected with an increased demand for zinc due to its function in enzymes required for cellular replication [1]. Zinc was found to be slightly mitogenic to epithelial cells *in vitro* at one zinc level (5 μg zinc/ml) [21]. However, we cannot exclude that zinc also influences the movement of epidermal cells over the wound bed although zinc treatment seemed to inhibit the migration of at least inflammatory cells in the dermis in

this study. In addition the wounds were kept moist and this ensures favorable conditions for epidermal cell migration [20]. Autoradiographic determination of incorporation of [^{3}H]-thymidine into DNA of the basal epithelial cells in wounds treated with or without zinc oxide could be one procedure to examine the mode of action *in vivo*.

Zinc oxide was found to be slowly but continuously ionized in serum *in vitro* [22], a result of its limited water solubility [23]. Our hypothesis is that this sustained release of zinc ions supplies an adequate amount of zinc for the cells to resurface the wounds more rapidly. The proper therapeutic range of the readily water soluble zinc sulfate is achieved only with difficulty and overdosing (toxicity) is possible. *In vitro,* a zinc ion concentration of 10 μg/ml arrested the proliferation of epithelial cells almost completely after 48 hr of exposure, whereas with a concentration of 6 μg/ml no toxicity was found [24]. Therefore, the dosing of zinc appears to be more crucial than the type of zinc salt applied.

Our results suggest that zinc is a pharmacologically active topical wound healing agent even in subjects with normal zinc status. The study has also demonstrated the advantage of zinc oxide over zinc sulfate, possibly because zinc derived from poorly water soluble zinc oxide is delivered continuously to stimulate re-epithelialization over an extended period of time. However, it remains to be investigated whether zinc influences the proliferative or migratory ability of epidermal cells in wound healing.

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