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LOCAL ABSORPTION OF ZINC FROM WOUNDS TREATED WITH VARIOUS ZINC-COMPOUNDS

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Abstract. It is shown that in the rat, zinc is absorbed from excisional wounds into serum, from various locally applied zinc oxide preparations as well as from zinc peroxide and zinc sulphate. The most pronounced absorption of zinc to serum was recorded from wounds treated with zinc peroxide. Microscopically the cellular inflammatory response observed in wounds treated with a gauze sponge impregnated with a zinc sulphate solution was weaker than that found with the other treatment methods used.

Zinc compounds have been used for centuries in the treatment of skin disorders. Of these, zinc oxide has been used most widely in dermatological and cosmetic medicaments (13). When using zinc pastes and powders, a drying effect on moist, inflamed areas has been achieved. The prevailing belief has been that zinc compounds are not absorbed after local application. However, no absorption studies have been performed on zinc oxide, perhaps due to the fact that no good and simple method for determination of zinc was available prior to the introduction of the atomic absorption technique about 20 years ago.

During treatment of excisional wounds on the rat with zinc tape, which contains zinc oxide in its adhesive substance, local absorption of zinc to the granulation tissue and serum has been shown (9). Local absorption of zinc has also been demonstrated during wound treatment with ^{65}Zn-labelled zinc sulphate in rabbits (18). The purpose of the present investigation was to investigate whether zinc is locally absorbed from a wound from different preparations of zinc oxide, zinc peroxide and zinc sulphate. As the concentration of copper in serum may be dependent on the type of local wound treatment (9), copper analyses have also been performed. In order to determine the tissue reaction after treatment with various zinc compounds during early wound healing, biopsy materials were taken from the wounds and subjected to light microscopy.

MATERIAL AND METHODS

Animals

Eighty-four male albino rats of the Sprague-Dawley strain weighing 250–400 g were used. The rats were housed in groups of 6 and fed a standard laboratory diet containing 50 ppm of zinc and tap water *ad libitum*. Seventy-four animals were operated on and divided into the following treatment groups: zinc peroxide (14 rats), zinc oxide powder (10 rats), zinc paste (10 rats), zinc tape (10 rats), zinc sulphate (10 rats), sterile gauze sponge (10 rats), plastic foil (10 rats). Ten animals served as controls and were not operated on.

Operations

Two circular wounds were made on the dorsolateral aspects of the backs.

The hair covering the prospective wound sites was removed with electric clippers and the outlines of the wounds marked with the end of an inked metal tube having a diameter of 4 cm. The excision was made through the panniculus carnosus to the fascia, using curved scissors. After wound retraction shortly after operation the longest and the shortest diameter of the wound were measured and the wound area calculated from the formula of an ellipse (8). The total wound area after wound retraction was 41.50 ± 0.34 cm^2 (mean $\pm$ S.E.M.; $n=74$). Treatment of the wounds was then begun.

Twenty-four hours later the animals of the different treatment groups were killed by decapitation under ether anaesthesia. The ten unwounded animals were killed at the same time. Before sacrifice the animals were starved for 24 hours. Samples of serum and pancreas were taken for zinc and copper analysis and samples from the wound and surrounding skin were taken for histological examination.

Wound Treatment Procedure

Preparations containing zinc oxide

Zinc oxide powder: 100% zinc oxide; *zinc paste:* 100 g paste contains 40 g zinc oxide and 60 g white petrolatum;

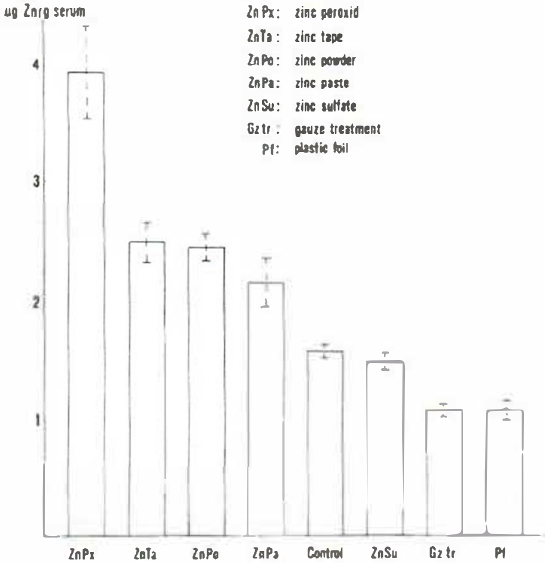

Fig. 1. Zinc concentration in serum (μg/g wet weight). The results are given as the mean±S.E.M. *t*-Test results: compared with controls:
ZnPx; ZnTa; ZnPo; Gztr; Pf: $p<0.001$. ZnSu–Pf: $p<0.001$
ZnPa: $p<0.01$. ZnSu–Gztr: $p<0.001$

zinc tape: the adhesive substance of the tape contains about 40% zinc oxide, 30% gum and 30% resin (Mölnlycke AB, Sweden). The zinc paste and the zinc oxide powder were applied in excess on the wound surface. The zinc tape was applied to the wound surface direct, covering the entire wound area.

Zinc peroxide

Fourteen animals were used (5 animals died during treatment). Before use, the zinc peroxide powder was sterilized and activated in an oven for 4 hours at 140°C and stored in dark-coloured bottles protected from light, at room temperature.

Immediately before application of the zinc peroxide suspension to the wound 25 g of zinc peroxide was suspended in 50 ml of a mixture of glycerol (25 g) and 3% hydrogen peroxide (25 g). A gauze pad soaked in zinc peroxide suspension was applied in excess to the wound surface.

Zinc sulphate

Zinc sulphate (0.2% solution) applied in excess to the wound surface by means of a gauze pad.

Reference treatments

Two methods of treatment without zinc were used. Either a sterile gauze sponge or sterile, plastic foil (Steridrape®) was placed on the wound surface.

Zinc and Copper Determination

Samples of serum and pancreas were taken for determination of zinc and copper concentrations as described earlier by Hallmans (8). The samples were placed in HCl-washed Phyrex tubes, weighed, dried, ignited and analysed by atomic spectrophotometry (Varian AA-6DB) as described by Bergman et al. (2). The zinc and copper determinations were performed at 213.9 nm and 324.7 nm, respectively.

Histology

Samples from the wounds and surrounding skin were fixed in 10% neutral formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin.

Statistics

The differences between group means for various variables were tested using Student's *t*-test for unpaired observations. The test was modified when the variances were found to differ significantly (*F*-test).

RESULTS

Zinc and copper concentrations

A pronounced increase was seen in the content of zinc in serum in the zinc peroxide treated animals after 24 hours (Fig. 1). During treatment with the different compounds of zinc oxide (zinc powder, zinc paste and zinc tape), there was also a significant increase in the serum concentration. The level of zinc in serum in the zinc sulphate treated animals did not differ from that of the control animals, whereas there was a significant decline in serum level of zinc in the groups treated with gauze and plastic foil. The serum level of copper differed only slightly among the various groups, showing a slight

Table I. *Copper concentration in serum (μg/g wet weight) and in pancreas (μg/g dry weight)*
The results are given as the mean±S.E.M.

	Control	Zinc peroxide	Zinc powder	Zinc paste	Zinc tape	Zinc sulphate	Plastic film	Gauze pad
Serum	1.52±0.07	1.36±0.08	1.44±0.05	1.74±0.05[b]	1.65±0.07	1.57±0.04	1.60±0.05	1.48±0.06
Pancreas	4.87±0.33	6.10±0.53[a]	4.29±0.52	5.68±0.68	5.14±0.40	5.69±0.65	6.34±0.70	5.43±0.42

[a] $p<0.05$, compared with controls.
[b] $p<0.001$, compared with controls.

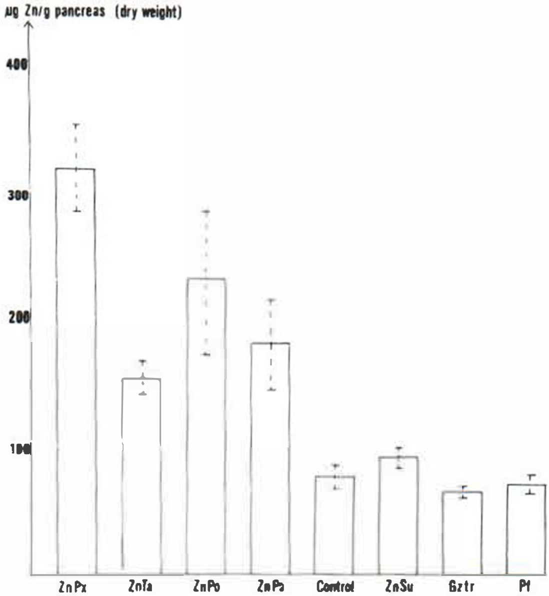

Fig. 2. Zinc concentration in pancreas (µg/g dry weight). The results are given as the mean±S.E.M. For explanation of symbols, see Fig. 1. *t*-Test results: compared with controls:

ZnPx; ZnTa: $p<0.001$ ZnSu–Gztr: $p<0.01$
Zn●; ZnP: $p<0.05$ ZnSu–Pf: $p<0.05$

decline in the zinc peroxide treated group and an increase in the zinc paste treated group (Table I).

In the pancreas, the concentration of zinc was found to be elevated in the zinc peroxide treated group and the various zinc oxide treatedgroups (Fig. 2). The increase was most pronounced in the first group. No differences were observed between the gauze and plastic foil treated groups, and the control group, though a decline was observed in both the gauze and the plastic foil treated groups when compared with the zinc sulphate treated group. An increase in copper concentration in pancreas related to controls was seen in the zinc peroxide treated group (Table I).

Histology

Treatment with both gauze pad and plastic foil provoked a moderate inflammatory reaction in the wounds. There was accumulation of neutrophil granulocytes and mononuclear cells interstitially in the oedematous tissue surrounding the wound, though predominantly in the wound surface. The blood and lymph vessels were dilated and leukocytic diapedesis was observed.

In principle, the same microscopic picture was seen in wounds treated with zinc peroxide and the various preparations of zinc oxide, though precipitates of foreign material, presumably the zinc compounds themselves, were seen surrounded by leukocytes near the wound surface (Fig. 3).

In wounds treated with a wet gauze pad soaked in zinc sulphate, the inflammatory picture was somewhat different. Very few inflammatory cells were seen near the surface, whereas more such cells were present some distance away, near the fascia of the underlying muscle. In the tissue surrounding the wound there was a pronounced oedema with markedly dilated vessels similar to the picture seen in the other treatment group (Fig. 4).

DISCUSSION

Zinc is an essential trace element for man and animals (15), is an integral part of several metalloenzymes (16), takes part in numerous stages in nucleic acid metabolism (11) and seems to be closely involved in the homeostasis of various inflammatory cells (3).

Clinical studies have indicated that the healing of venous leg ulcers (7) as well as ischemic leg ulcers (6) is impaired by zinc deficiency. These studies have also shown that oral supplementation of zinc to zinc-deficient patients has a beneficial effect on the healing time of the ulcers. During oral treatment of zinc there seems to be a lag period of some weeks (2–7 weeks) before any effect ofthe treatment on the wound healing process makes its appearance (14, 6).

In spite of the fact that zinc oxide has been used extensively, principally within the field of dermatology during this century, it has not yet been determined whether or not zinc is absorbed through the epidermis itself, or via defects in the epidermis, Zinc oxide in pastes and powders has been thought to have only a local, drying effect on various skin disorders (17). Zinc peroxide has been used as an antiseptic agent to obtain a slow release of oxygen from wounds infected with anaerobic bacterias (17, 10). Zinc sulphate on the other hand has clinically been used for treatment of conjunctivitis.

The present results demonstrate that zinc is absorbed from various zinc oxide preparations as well as from zinc peroxide and zinc sulphate applied direct to excisional wounds in the rat. The most pronounced absorption of zinc to serum was recorded from wounds treated with zinc peroxide.

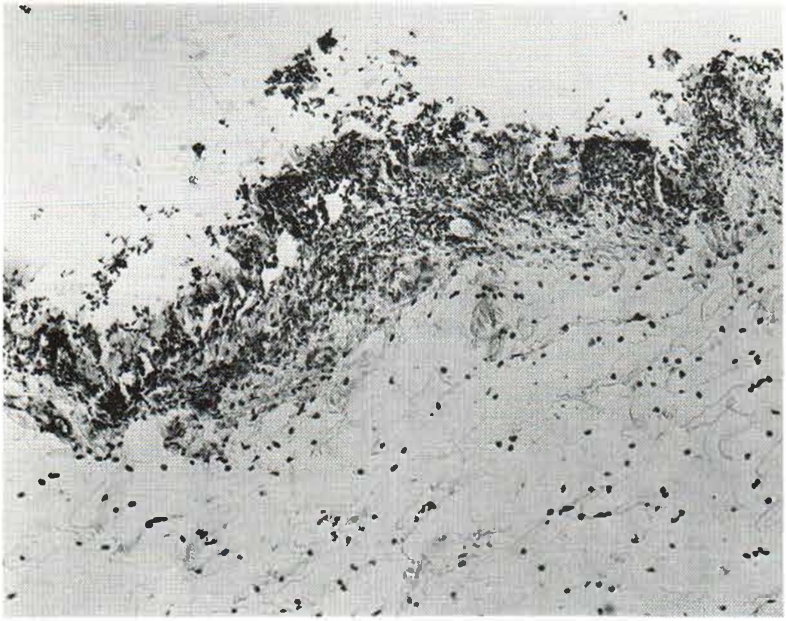

Fig. 3. Zinc peroxide treated wound. Precipitates of zinc peroxide heavily infiltrated with leukocytes are seen on the surface. Beneath the surface the tissue is edematous and invaded by inflammatory cells. Hematoxylin-eosin. ×130.

During wound treatment with zinc peroxide, oxygen is slowly released and a mixture of zinc oxide and zinc hydroxide is formed (12). This reaction obviously creates a favourable condition for local zinc absorption, compared with treatment with zinc tape, zinc powder and zinc paste, from which less zinc is absorbed locally, even though zinc oxide is present in large amounts on the wound surface. During treatment with zinc peroxide, 5 animals died. At present we have no certain explanation why this happened. It may be that a sudden, toxic overload of zinc together with the operation trauma caused the death of the animals.

There was also a local absorption of zinc from the wound sites treated with zinc sulphate. While these animals had relatively unchanged levels of

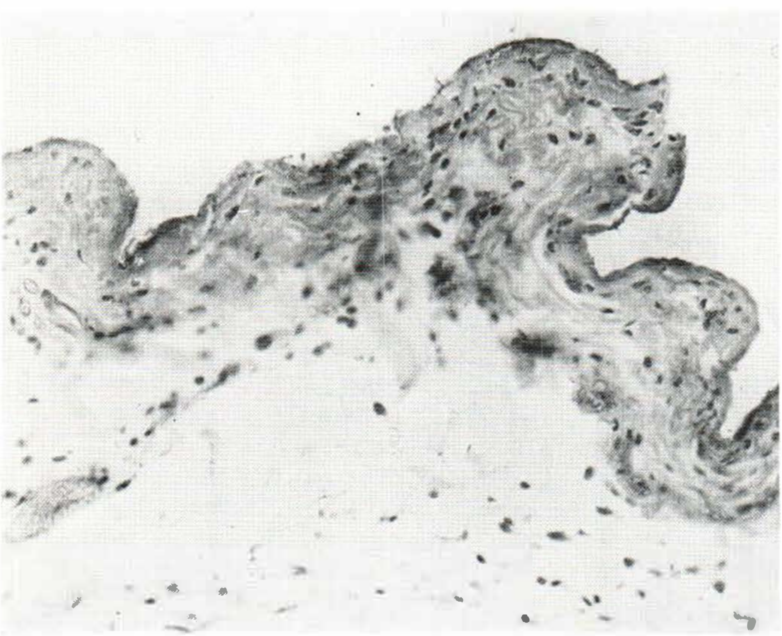

Fig. 4. Zinc sulphate treated wound. Dilated vessels are seen near the surface. Very few inflammatory cells are seen in the edematous tissue. Hematoxylin-eosin. ×240.

zinc in serum and pancreas compared with controls, they also had higher levels of zinc in serum and pancreas when compared with operated animals treated with gauze pad or plastic film. These results are in agreement with those presented by Yankell (18) who demonstrated that ^{65}Zn-labelled zinc sulphate was absorbed from wounds in rabbits. Probably, the amount of absorption of zinc in ionic form is only a matter of concentration of the zinc sulphate in the solution applied.

In animals with wounds treated with gauze pad or plastic foil, the serum levels of zinc were reduced significantly, compared with each of the zinc-treated groups and with control animals. This reduction in serum zinc may have been caused by a redistribution of loosely bound zinc, as has recently been demonstrated during acute inflammatory conditions in the rat (1).

Compared with the dramatic changes in zinc concentrations, the copper levels in serum remained fairly stable in the different groups. In the zinc peroxide treated group there was, however, a slight decline in copper concentration and in the zinc paste treated group an increase was found. The fall in the level of serum copper in zinc peroxide treated animals might have been caused by the high levels of serum zinc, as high levels of zinc in the diet are known to depress the serum level of copper in rats (5).

Microscopically, a surprisingly weak cellular inflammatory response was seen in wounds treated with gauze pads soaked in zinc sulphate solution, compared with wounds treated with different kinds of zinc oxide, zinc peroxide, plastic film or pure gauze pads. Whether the inflammatory response is alleviated by the zinc sulphate on the wound surface or by the moisture or other factors is uncertain. However, evidence is accumulating that zinc ions may inhibit certain cell functions including phagocytosis by both macrophages and neutrophilic leukocytes (3), the two most important cells involved in the acute inflammatory cell response.

The basic conclusion of the present study is that zinc is absorbed locally from wounds from different kinds of zinc compounds. Clinical experience of adhesive zinc tape treatment of venous leg ulcers has previously shown it to be beneficial (4). Through local administration of zinc the zinc requirements within the wound may be quickly satisfied. Sufficient quantities of zinc would then become available for the wound healing process during the first days of treatment, even though body stores may be low. This may be beneficial for the wound healing process, and it is now in any case time to look at the biological effects of locally applied zinc. At present, experiments are also in progress to find out whether or not local absorption of zinc can also be achieved through partly or wholly intact epidermis.

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