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Release and Absorption of Zinc from Zinc Oxide and Zinc Sulfate in Open Wounds

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The pharmacokinetic behaviours of zinc oxide and zinc sulfate when applied as single doses to full-thickness excised rat skin wounds were studied. In the zinc oxide group, the wound fluid zinc concentration increased slightly over the 48-h postoperative period due to increased solubilization of zinc oxide, attributed to increased protein concentration of the wound fluid. When zinc sulfate was applied to the wounds, the wound fluid zinc concentration decreased rapidly during the first 4 postoperative h and then at a slower rate. The changes in the serum zinc level followed essentially the same kinetic pattern as that of the wound fluid zinc levels. The zinc concentration of the wounded tissue remained almost constant in zinc oxide treated wounds whilst it diminished in zinc sulfate treated wounds. In conclusion, zinc oxide delivers zinc ions to wounds over an extended period of time which results in constant wound tissue zinc levels. In contrast, zinc sulfate rapidly delivers zinc ions which results in decreasing tissue zinc levels. ***Key words: Pharmacokinetics; Rat wounds; Solubility, Zinc oxide.***

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Zinc treatment is believed to be effective for wound healing only in zinc-deficient patients (1). However, we have recently shown that topical zinc accelerated re-epithelialization of partial-thickness skin wounds in nutritionally balanced pigs, but only when zinc was administered in the chemical form of zinc oxide (ZnO) and not in the form of zinc sulfate ($ZnSO_4$) (2). We hypothesized that the beneficial effect of ZnO was due to its slow dissolution rate which would result in a continuous delivery of zinc ions to the wounds over an extended period of time (2). $ZnSO_4$, on the other hand, promptly dissolves in aqueous media, such as wound fluid, and therefore probably delivers zinc ions more rapidly after its application to the wound. It would thus be of interest to know the zinc levels in wounds after topical

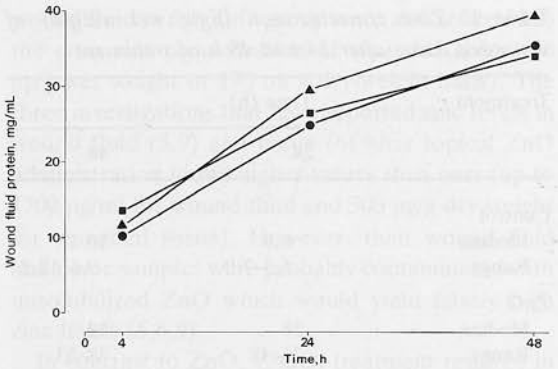

Fig. 1. The changes of wound fluid protein concentration over time. Each symbol represents the average of six measurements. Only the median values are included for reasons of clarity. Control (●), ZnO (▲), $ZnSO_4$ (■).

treatment with ZnO and $ZnSO_4$. However, reliable information about local zinc concentrations after zinc application to wounds is scarce, although there is more data on the systemic absorption (3–6).

In this descriptive study we compared the kinetics of zinc delivery from ZnO and $ZnSO_4$ after single applications to wounds over a 48-h treatment period.

MATERIALS AND METHODS

Dressings were prepared by impregnating absorbent gauze with ZnO and $ZnSO_4$ as described in detail elsewhere (2). Control dressings were only impregnated with the binding agent (polyvinyl pyrrolidon, PVP) of the zinc compounds. The zinc content of the ZnO dressing was 250 μg zinc/cm^2 or 14 mg zinc/g, and of the $ZnSO_4$ dressing 66 μg zinc/cm^2 or 3 mg zinc/g. The PVP content of the dressings was 5 mg/g.

The rat was chosen as the experimental animal because most of the information available on zinc absorption from wounds has been derived using the rat (3,4,6). The full-thickness skin excision was used as a wound model because preliminary experiments showed that the partial-thickness wound produced insufficient quantities of wound fluid for zinc determinations. Twenty-seven male albino Sprague-Dawley rats (210–250 g; ALAB, Sweden), 3 for each time point and dressing type, were used. The rats were kept in individual Makrolon® cages and fed standard pellets containing 170 μg zinc/g (R3, Astra-Ewos, Sweden) and tap water (< 0.1 μg zinc/ml) *ad libitum*. Two circular full-thickness skin wounds (each 11 cm^2) were made on either side of the spine in the thoracic-lumbar region in anesthetized rats (4). The same type of dressing (15 cm^2) was applied to the two wounds in each rat. In a previous study, where ZnO was found to have a beneficial effect on wound healing, dressings were covered with a semiocclusive polyurethane membrane (Tegaderm®, 3M, MN, USA) and moistened with saline (1.5 ml at 37°C) (2). We adopted this procedure and it also enabled us to obtain sufficient amounts of wound fluid for the zinc analyses. An adhesive tape (Durapore®, 3M) was wrapped twice around the trunk to secure and protect the dressings. No dressing changes were carried out. The rats were killed after 4 (n=9), 24 (n=9) and 48 (n=9) h of treatment by heart puncture and exsanguination under anesthesia.

Serum was obtained after centrifugation at 1000 × g for 10 min. Dressings were put into a 5-ml plastic syringe with the plunger removed, then the plunger was inserted and the wound fluid (≈0.5 ml) was squeezed out of the moist dressings. Finally, wound fluid samples were clarified through 0.22 μm filters mounted on the tip of the syringe primarily to remove unsolubilized ZnO particles and thus leaving only solubilized ZnO. The average particle size of ZnO powders derived from the French process has been estimated to 0.34–0.99 μm (7).

To get a rough estimate of the total quantity of zinc delivered to the wounds from the ZnO and $ZnSO_4$ dressings, the dressings were analyzed for their zinc content before and after 48 h of treatment.

Wounded tissue, i.e. inflamed connective tissue, was dissected free from underlying fascia and rinsed with ice cold deionized water to remove superficial zinc contaminants. Studies of the wounded tissue through a dissecting microscope showed no ZnO aggregates after ZnO treatment.

Zinc and protein analyses

Zinc analyses of diet, tap water, dressings, serum, wound fluid and wounded tissue were performed with atomic absorption spectrophotometry according to procedures previously described (2,4).

The intraindividual variability of the zinc concentrations of the wound fluid and wounded tissue was estimated. For each animal, the difference between the zinc concentrations obtained for the two wounds was divided by the average of the two concentrations. The average variability for the wound fluid for control treated wounds was 16%, 12% for ZnO treated wounds and 32% for $ZnSO_4$ treated wounds; the corresponding values for the wounded tissue were 16%, 53% and 74%, respectively. The variability of duplicate analyses of wound fluid was likewise determined to 2%.

Protein concentration of wound fluid and serum was determined according to Ohnishi & Barr (8). The variability of wound fluid protein concentration, determined as described in the paragraph above, was 12% overall.

Data processing

To give maximum information, and also because an assumption of normal distribution cannot be made, we preferred to present the data as median values and ranges, without testing for significance.

RESULTS

Dressings remained moist throughout the 48 h experimental period and thus were easy to remove. The protein concentration of the wound fluid increased over the experimental period (Fig. 1) and was about 70% of that of serum at 48 h.

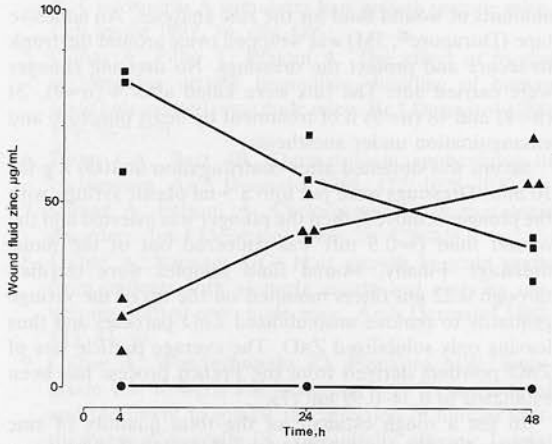

Fig. 2. The changes of wound fluid Zn concentration over time. Each symbol represents the average zinc concentration of the two wounds on each rat. For the control group only the median values are included for reasons of clarity. The median zinc concentration at time 0 h (obtained by zinc analysis of saline moistened dressings before application to the wounds) was 0.0 µg/ml for the control group, 29 µg/ml for the ZnO group and 490 µg/ml for the $ZnSO_4$ group. Control (●), ZnO (▲), $ZnSO_4$ (■).

The wound fluid zinc concentration remained nearly constant over time in control treated wounds (0.8 at 4 h, 0.7 at 24 h and 1.1 µg/ml at 48 h). In the ZnO group the wound fluid zinc remained fairly constant, although it increased to about 55 µg/ml at 48 h. A converse kinetic pattern was found in the zinc sulfate group where the wound fluid zinc decreased during the entire 48-h experimental period with the sharpest decline occurring within the first postoperative 4 h (Fig. 2). About 450 µg zinc (12% of the initial dosage) was delivered to each wound from the ZnO dressing and about 650 µg zinc (65% of the initial dosage) from the $ZnSO_4$ dressing over the 48-h treatment period. The zinc concentration of the wounded tissue was almost at the same level in ZnO treated wounds 24 and 48 h postoperatively, whereas it decreased in $ZnSO_4$ treated wounds (Table I).

The serum zinc level was elevated in zinc treated groups at 4 and 24 h, whereas at 48 h it appeared to be higher than controls only in the ZnO group (Table II).

DISCUSSION

Zinc absorption from topically applied zinc compounds has been studied predominantly by measuring the absorption through wounds into the body (3–6). The focus of this investigation was to compare the local zinc concentrations over time after a single application to wounds of the virtually water-insoluble ZnO and the highly water-soluble $ZnSO_4$.

In accordance with earlier findings (3–6), zinc was absorbed through the wounds as evidenced by increased serum zinc levels in zinc treated rats. In ZnO treated rats, the increase prevailed throughout the 48-h treatment period. The slight increase in serum zinc from 24 to 48 h in the ZnO group was most likely due to the increased wound fluid zinc level. At 48 h the wound fluid zinc (55 µg/ml) was twofold the solubility of ZnO in saline (pH 7.4, 37°C) (4) which could be explained by increased solubilization of ZnO due to the complex formation between zinc and proteins in the wound fluid.

The relatively constant zinc levels in serum and

Table I. *Zinc concentration (µg/g wet weight) of wounded tissue after 24 and 48 h of treatment*

Treatment	Time (h)	
	24	48
Control		
Median	6.9	10.4
Range	5.3–7.0	9.6–12.2
ZnO		
Median	38	42
Range	36–48	38–53
$ZnSO_4$		
Median	204	84
Range	92–308	51–86

Table II. *Serum zinc levels (µg/ml) after 4, 24 and 48 h of treatment*

Treatment	Time (h)		
	4	24	48
Control			
Median	1.1	1.2	1.2
Range	1.0–1.2	0.8–1.3	1.1–1.4
ZnO			
Median	1.5	1.3	1.5
Range	1.4–1.7	0	1.4–1.6
$ZnSO_4$			
Median	2.4	1.4	1.2
Range	1.9–2.6	1.4–1.5	1.2–1.4

wound fluid in the ZnO group were also reflected in the even zinc concentration of wounded tissue (40 μg/g wet weight or 170 on a dry-weight basis). The three investigations that have reported zinc levels in wound fluid (5,9) and tissue (6) after topical ZnO administration found higher values than ours (up to 1700 μg/ml for wound fluid and 300 μg/g dry weight for wounded tissue). However, their wound fluid and tissue samples were probably contaminated with unsolubilized ZnO which would yield falsely high zinc levels (5,6,9).

In contrast to ZnO, $ZnSO_4$ treatment resulted in declining zinc concentrations in wound fluid, serum and wounded tissue, although the zinc concentration in the wounded tissue remained higher than ZnO treated wounds at 48 h. Although high initial zinc concentrations were observed with the $ZnSO_4$ gauze dressing, this did not inhibit re-epithelialization which, however, both lower and higher concentrations of $ZnSO_4$ in the same vehicle did in another study (2). Furthermore, $ZnSO_4$ in a slow-release vehicle applied daily to wounds promoted granulation tissue formation despite the high zinc concentration in the granulation tissue (250 μg/g wet weight) (10).

In summary, when ZnO is applied to wounds in the amount and form used in this investigation it results in a sustained delivery of zinc ions, and the local zinc concentration remains fairly constant during at least a 48-h treatment period. In contrast, when $ZnSO_4$ is used, the local zinc levels diminish over the treatment period. The observed pharmacokinetic patterns might explain the differences in the effect between ZnO and $ZnSO_4$ on wound healing (2).

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