Influence of Two Vehicles for Zinc Oxide on Zinc Absorption Through Intact Skin and Wounds

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The zinc absorption from zinc oxide applied on intact and on wounded skin were compared using two vehicles: one containing gum rosin and the other containing hydrocolloids. After treatment of intact human forearm skin for 48 h, the zinc concentration was more than two-fold higher in the epidermis and interstitial fluid beneath the rosin vehicle than beneath the hydrocolloid vehicle. Systemic absorption of zinc through full-thickness rat skin wounds treated with zinc oxide increased significantly, however, with the use of the hydrocolloid vehicle. In vitro experiment indicated that the solubility of zinc oxide in buffered saline (pH 7.4) increased in the presence of hydrocolloids. The study, thus, indicates that rosin in combination with zinc oxide enhances the transport of zinc through intact human skin, and that hydrocolloids promote the zinc absorption through wounds. *Key words: Percutaneous absorption; Human skin; Gum rosin; Zinc resinate.*

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In topical formulations zinc oxide (ZnO) alone or in combination with other active substances, e.g. corticosteroids, are used for treatment of skin disorders (1). Zn absorption from topical ZnO has been investigated thoroughly using an occlusive dressing (Mezinc®) with ZnO incorporated in the adhesive (2). Experiments with rats have demonstrated that Zn from Mezinc® is absorbed through both intact and broken skin in the form of Zn ions (2). Furthermore, when ^{65}Zn-labelled Mezinc® was applied on excised wounds the ^{65}Zn activity in blood was about 100-fold than when it was applied on the same surface area of intact skin in rats indicating a slower absorption rate of Zn through intact skin (2). In humans, increased serum Zn levels were found after Mezinc® treatment of extensive burn wounds (5–20% of the body surface area) indicating Zn absorption through the wounds (2, 3). Recently, it was shown that Zn was transported through intact human skin from Mezinc® (4). However, Mezinc® contains gum rosin (colophony), which can cause contact allergy, whereas hydrocolloid dressings, for example, do not cause adverse skin reactions to the same extent (5).

The aim of this investigation was to compare the Zn absorption from ZnO in a hydrocolloid vehicle with that of Mezinc® when applied topically on healthy human skin and on skin wounds in rats.

MATERIALS AND METHODS

Percutaneous Zn absorption study in humans was carried out by raising suction blisters on treated skin and analyzing their Zn content (4). Five healthy volunteers (4 females, 1 male) aged 23–41 years participated in the study after giving their informed consent. The adhesive of Mezinc® (Mölnlycke AB, Mölnlycke, Sweden) is composed of Portuguese gum rosin (35%, w/w), natural rubber and white mineral oil as well as ZnO (25% or 2.7 mg Zn/cm^2). ZnO (Merck, pro analysi) was mixed with the hydrocolloids (carboxymethylcellulose sodium, pectin and gelatin) and synthetic rubber (polyisobutylene) solely for the purpose of this study. The maximum amount of ZnO that could be incorporated without substantially altering the physical properties of the hydrocolloid was 6% (w/w) of ZnO (or 6.0 mg Zn/cm^2). The two vehicles were laminated with polyvinylchloride (Mezinc®) or with polyurethane (ZnHCD) backings to adhesive and occlusive dressings. Measured with an evaporimeter (ServoMed) the water vapor permeability rates through the dressings when applied on normal skin of the forearm were: uncovered skin: 8.0 g $H_2O/m^2/h$, Mezinc®: 2.5 g $H_2O/m^2/h$, HCD: 2.2 g $H_2O/m^2/h$ (Ågren, unpublished observations). The volar forearm skin (about 10 cm from the wrist) was treated with one disc of each of the ZnO-supplemented dressings (8 cm^2) placed 5 cm apart on the left arm. After 48 h of treatment, suction blisters on treated skin were raised by applying suction cups (Dermovac®) as described earlier (4). Since much Zn is retained by the stratum corneum (4) the procedure was modified to include 10 successive tape strippings (Scotch™ Magic 810, 3M) of treated skin preceding the blistering. The blister dome (epidermis) and blister fluid (interstitial fluid) were then analyzed for their Zn content (4).

A complementary experiment was performed to study the release of Zn to the uppermost epidermis by measuring the total amount of Zn obtained in 10 tape strippings. The two subjects showing the largest difference in Zn level of

Table I. *Zn concentration in epidermis and blister fluid after 48 h of treatment with ZnO in two different vehicles (ZnHCD and Mezinc)*

Subject No.	Age (years)	Epidermis (μg/g dry weight)		Blister fluid (μg/ml)	
		ZnHCD	Mezinc	ZnHCD	Mezinc
1	23 F	57	217	0.26	0.70
2	28 F	36	120	0.27	0.86
3	33 F	61	104	–	–
4	35 M	45	92	0.40	0.75
5	41 F	45	103	0.23	0.39
Mean ± SEM		49±5	127±26*	0.29±0.04	0.68±0.10*

* $p < 0.05$, paired t-test.

the epidermis between the two dressings were selected for this experiment. After treatment of the forearm skin for 48 h, the treated skin site (= 8 cm^2) was stripped twice to remove non-solubilized ZnO before measuring the 10 successive tape strippings on zinc.

Zn absorption through open wounds was investigated in an experiment on rats because relatively large wounds can be inflicted on the animals without severely affecting their general health and thus enabling the recording of systemic Zn absorption without the need of radiolabelled Zn.

Male Sprague-Dawley rats (200–290 g) were anesthetized with a mixture of intramuscular fentanyl citrate (0.1 mg/kg body weight) and droperidol (5.0 mg/kg). Two full-thickness skin excisions each measuring 10 cm^2 and extending to the fascia were made on the shaved back of each rat. The total wound area corresponds to about 8% of the body surface area. Five rats were treated with the plain hydrocolloid (HCD), 5 with ZnHCD and 5 with Mezinc®. Gauze sponges were placed on the dressings and an adhesive tape was wrapped around the animals. After 24 h the animals were killed, and serum and liver were analyzed for their Zn content (4). Microscopical examination was carried out on wound specimens stained with Hematoxylin-eosin.

To test the hypothesis that the hydrocolloid vehicle by itself increased the aqueous dissolution of ZnO, an in vitro experiment was carried out. ZnO (Merck, pro analysi) alone (1.2 g/l) or the same amount of ZnO in ZnHCD was introduced into ampoules containing 80 ml of physiological saline (0.9% w/v NaCl) in 0.1 M HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pKa 7.55) buffer (pH 7.4) under N_2 to prevent the formation of carbonates. The ampoules were sealed, and shaken continuously for 24 h in a water bath maintained at 37°C. The experiment was performed in triplicate. The suspensions were passed through 0.22 μm filter (Millipore®) and the Zn content was determined.

Zn analyses were carried out with atomic absorption spectrometry according to procedures previously described (4).

Statistical evaluations were made with the t-test and $p<0.05$ was considered as statistically significant.

RESULTS

Percutaneous absorption

Both dressings remained intact without leaving visible adhesive residues on the skin or causing unfavorable skin reactions after removal. The Zn levels in both epidermis and blister fluid were about twice as high after Mezinc treatment than after ZnHCD treatment (Table I).

In the complementary experiment, 1.6 μg of Zn was found in the 10 tape strippings of the skin treated with ZnHCD and 1.4 μg of Zn with Mezinc in Subject 1. The corresponding figures for Subject 2 were 2.6 and 2.5 μg of Zn, respectively. Negligible quantities of Zn were recovered from adjacent stripped untreated skin.

In Subject 4, ZnHCD was also compared with the plain hydrocolloid vehicle under the same experimental conditions as in the main study. The blister fluid Zn level was 0.49 μg/ml beneath HCD and 0.45 μg/ml beneath ZnHCD, indicating lack of percutaneous Zn absorption from ZnHCD.

Table II. *Zn concentration in serum and liver in treated rats 24 h post-operatively*

	HCD (n = 5)	ZnHCD (n = 5)	Mezinc (n = 5)
Serum (μg/ml)	0.95±0.02[a]	4.06±0.48***	2.56±0.20***
Liver (μg/g dry weight)	146±2	390±23***	206±11**

** $p < 0.01$, *** $p < 0.01$ compared with the HCD group.
[a] Mean ± SEM

Wound absorption

In the open wounds on rats, the adhesive of the hydrocolloid dressings disintegrated, whereas Mezinc remained macroscopically intact. As revealed by the increased Zn concentrations of serum and liver systemic absorption of Zn through open wounds in the rats increased after treatment with both the ZnO supplemented dressings (Table II). Absorption was further enhanced with ZnHCD as compared with Mezinc ($p<0.05$ for serum, $p<0.01$ for liver) (Table II). The inflammatory cell infiltrate, composed mostly of polymorphonuclear leukocytes, was mainly found near the wound surface, although a slight involvement of the superficial muscular tissue was seen in all treatment groups. The wounded tissue in the hydrocolloid groups was conspicuously edematous compared with the Mezinc group. No foam cells were seen in any treatment group.

In vitro, it was found that more ZnO was solubilized with hydrocolloid (67 μg/ml) than without (28 μg/ml) in buffered saline.

DISCUSSION

An earlier study showed that Zn concentrations in epidermis, blister fluid and dermis beneath a ZnO medicated dressing (Mezinc) were higher than those beneath the Mezinc vehicle only containing rosin, mineral oil and natural rubber (4). Here Mezinc was compared with ZnO in a vehicle (ZnHCD) containing hydrocolloids (pectin, gelatin and carboxymethylcellulose) and synthetic rubber. After 48 h of treatment the Zn levels in epidermis and blister fluid beneath Mezinc were significantly higher than beneath ZnHCD (Table I). The same magnitude of difference regarding the Zn levels found earlier between Mezinc and its vehicle (4) was also found here between Mezinc and ZnHCD indicating no or minimal percutaneous zinc absorption from ZnHCD applied on intact human skin.

It is difficult to comment on the cause/causes of the enhanced skin penetration of Zn with Mezinc rather than with ZnHCD. However, about equal amounts of solubilized ZnO were found in the upper layers of stratum corneum beneath the two dressings. This finding indicates a similar release of Zn from the two dressings to the skin. ZnO forms Zn soaps with rosin acids – the Zn resinates – which are almost insoluble in water but more soluble in nonpolar (organic) solvents, e.g. octanol (6). A generally accepted theory is that a drug's ability to penetrate skin is governed, apart from its molecular weight and water solubility, by the octanol/water partition coefficient (7). Theoretically, then, the inorganic ZnO can be converted into organic Zn resinates in Mezinc (8) and these are able to penetrate the whole skin. Zn stearate, another example of a Zn soap, is not allowed for use on skin in some countries due to the risk of absorption (9). Rosin may also increase the permeability of the skin per se thereby enabling Zn transportation through the skin. The disadvantage of rosin is that it is a skin sensitizer, although, it has been shown that allergenicity is lower with purified resin acids, which make up about 90% of rosin, compared with crude rosin (10).

Opposite results were found when the two ZnO dressings were applied on wounded skin. In this case the hydrocolloid vehicle seemed to promote Zn absorption from ZnO in wounds in comparison with Mezinc (Table II). In another study, Zn absorption from ZnO powder alone or ZnO in petrolatum (40%) applied "in excess" on wounds in rats was compared with Mezinc (2). Hallmans (2) did not find any significant difference in the serum Zn level between the three ZnO formulations after 24 h of treatment although serum Zn was increased in the ZnO-treated groups compared with non-Zn-treated groups (2). Zn absorption from these wounds correlates positively with the Zn ion concentration applied (2). Thus, a possible explanation for the increased absorption with ZnHCD is that the ionization of ZnO with hydrocolloids increased in the wounds as found in vitro.

In conclusion, the ZnO vehicle is an important determining factor for Zn delivery through intact and to wounded skin.

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A Possible Role for Superoxide Production in the Pathogenesis of Contact Dermatitis

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Superoxide generation by blood monocytes was examined in patients with irritant, nickel, and chromate hand dermatitis. Phorbol myristate acetate stimulated monocytes generated significantly more superoxide in patients with nickel dermatitis, as did patients with hand eczema generally. No significant stimulation of monocyte superoxide generation occurred with either opsonized zymosan or PMA in the presence of excess superoxide dismutase in any of the groups of hand dermatitis. The results indicate a biochemical stimulation of superoxide which may accentuate the immunological damage in the skin that is observed in nickel and perhaps chromate dermatitis.

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Oxygen-derived free radicals (ODFR) are chemical species which owe their reactivity to the possession of an unpaired electron in their outer orbit. Free radicals are known to play an important role in normal cellular metabolism (1), but there is increasing evidence for their involvement in tissue damage (2). Phagocytes such as neutrophils, tissue macrophages and blood monocytes (3–5) possess the capacity to produce large quantities of ODFR and metabolites via the initial production of the superoxide anion. It is suggested that these ODFR may be important initiators of both acute and chronic inflammatory reactions (6, 7).

Miyachi et al. (8) examined the potential role of ODFR in chronic cement dermatitis. They found that stimulation of polymorphonuclear leukocytes in patients suffering from chronic cement dermatitis generated markedly increased levels of superoxide anion, while there was only a slight increase in cells from cement workers without dermatitis. They concluded that although cement dermatitis is initiated as a contact sensitivity to chromate, it is possible that the inflammatory process is exacerbated by tissue damage from ODFR. In chronic eczema, it is known that there is an increased production of monocytes in the patients' bone marrow, possibly as a result of monocyte recruitment at the site of inflammation (9). Therefore, in the present study we have examined, in patients with both allergic contact and irritant contact dermatitis, oxygen-derived free radical generation by monocytes, which are cells of primary importance in both the induction and mediation of the tissue response in allergic contact dermatits (10).

PATIENTS AND METHODS

Patients

Seven patients (3 males, 4 females, age range 16–45 years) with irritant contact dermatitis on the hands and who were patch test negative to the ICDRG standard series of contact allergens (11) were studied. All had dermatitis on the backs and sides of fingers. These patients had no history of atopy or other significant medical illness.