Topical Zinc Sulfate Augmentation of Human Delayed Type Skin Test Response

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The ability of topical zinc sulfate to augment the cutaneous delayed hypersensitivity response to Candida antigen was evaluated in 47 adults (15 controls and 32 hospitalized patients). On each adult subject, intradermal standard Candida extract was administered to each forearm followed immediately by topical application of 10% zinc sulfate in Aquaphor® ointment on one arm and Aquaphor® alone on the other arm. The reaction size was assessed in a single blinded manner. Of the 24 subjects who reacted positively to Candida antigen, a significantly larger ($p<0.01$) number of individuals showed an augmented cutaneous delayed hypersensitivity response in the arm on which the topical zinc sulfate had been applied. However, when patients were stratified by plasma zinc concentrations, only the normal plasma zinc patient group demonstrated a statistical augmentation. Possible mechanisms and selectivity of this observed effect are discussed. These findings suggest a role for topical zinc application to augment cutaneous immune responsiveness.
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Zinc is an important trace element that is involved in immunological responsiveness. As a cofactor in many enzymes, zinc deficiency may be associated with diminished lymphokine production (1). In animals several aspects of cell mediated immune response have been shown to be affected by zinc restriction. These effects include diminished delayed hypersensitivity skin reactions and natural killer cell function, thymic involution, and decreased antigen specific antibody response (2). In humans, zinc deficiency is thought to play a role in the immunodeficiency state associated on occasion with acrodermatitis enteropathica (3).

It is also clear that zinc can act as a mitogen causing nonspecific B lymphocyte activation in vitro (4). Furthermore, oral zinc sulfate administration in healthy volunteers results in enhanced in vitro lymphocyte response to typical mitogens compared with those in a matched untreated control group (5). Thus, augmentation of immune responsiveness is not limited to zinc deficient states.

One study demonstrated topical zinc sulfate ointment augmentation of delayed type skin tests responses to Candida extract in children who were protein malnourished and who had low plasma zinc concentrations (6). In that study there was a significant negative correlation between skin test augmentation by topical zinc and plasma zinc concentrations. Because this observation showed that antigen specific skin test responses were increased with topical zinc sulfate, there exists the possibility of use of topical zinc to enhance diagnostic sensitivity with skin testing, or to augment T-cell mediated inflammatory response in cutaneous tumors or infections in zinc deficient, malnourished patients. Thus we studied a group of adult hospitalized patients and healthy subjects with 2 objectives: 1) to examine the general utility of topical zinc application in delayed type skin

testing and, 2) to examine correlations between topical zinc skin test enhancement and plasma zinc concentration.

MATERIALS AND METHODS

Participants consisted of healthy volunteers and medical in-patients with various illnesses from University Hospital, Newark, New Jersey. Informed consent was obtained according to the protocol approved by the institutional review board. Thirty-two patients and 15 healthy volunteers were treated.

The study design involved pre-test determinations of non-fasting morning plasma zinc and copper concentrations. Skin testing consisted of an intradermal injection of 0.1 ml of a 1 : 1000 (weight : volume) aqueous extract of Candida albicans (Cooke Institute of Allergy, N. Y.). The skin tests were bilaterally administered on the volar surface of the forearm or the lateral surface of the upper arm.

A control phenol buffered saline injection was simultaneously placed in the same areas but at least 5 cm away from the Candida skin test sites. On one arm both the control and Candida skin test sites were treated with 10% zinc sulfate in Aquarphor® ointment applied as a thin layer of approximately 2×3 cm area and then covered with a Band Aid®. On the other arm both sites were similarly treated with Aquaphor® ointment without zinc sulfate. The ointments were reapplied at 24 hours with change of Band Aids®. At 48 hours the ointments were removed, and any area of skin test induration was measured in two perpendicular dimensions in millimeters by an observer unaware of topical zinc location. This area was expressed as the product of the two dimensions. All subjects tested had plasma zinc and copper determinations performed by flame atomic absorption spectrophotometry, as previously described (7). Patients' medications, diagnoses, serum albumin, and hemoglobin were noted at time of skin tests.

RESULTS

Of 32 patients tested, 15 had positive Candida skin test responses. Their ages ranged from 44 to 83 years of age (Table I). Of the 15 healthy subjects tested, 9 reacted. Their ages ranged from 23 to 40 years of age. None of the reactors were taking immuno-suppressant mediction; the controls were taking no medications. None of the tested individuals were taking supplemental zinc. No reactions were seen at the control phenol buffered saline injection sites with or without zinc ointment. The male : female ratios for responding controls (5: 4) and patients (9: 8) were comparable.

Eight patients reactors had plasma zinc concentrations of less than 70 μg/dl (our lower limit of normal). The plasma zinc concentrations for this group ranged from 36 to 68 μg/dl (Table II). The remaining 7 skin test reactive patients had normal zinc levels (72–90 μg/dl). The diagnoses of the low zinc group included cardiovascular disorders (3), neoplastic disease (2), infectious disease (2), and cirrhosis (1). The normal zinc group diagnoses consisted of cardiovascular disorders (4), gastrointestinal hemorrhage (1), alcoholism (2) and anemia (1).

Of the 24 individuals with positive skin test reactions, 19 showed enhanced skin test reactivity with topical zinc application (zinc ointment reaction greater than zinc free ointment reaction), 4 showed enhanced reactivity with zinc free ointment (zinc free ointment reaction greater than zinc ointment reaction), and 1 had equivalent reactions on both arms. Analyses of this data by binomial proportion testing showed a statistically significant enhancement with topical zinc ($p<0.01$). In addition, paired t testing for skin test reactions in the entire group of patients and normals showed a significant increase in zinc applied skin test reactions ($t=2.065$, $p<0.05$) with a mean increase of 115.4 ± 57.4 mm^2.

In the group of patients with low plasma zinc concentrations ($n=8$), a very variable response (4 with zinc augmentation, 3 with zinc free augmentation, and 1 equivalent

reaction) was observed with a mean difference in area (between zinc and no zinc reaction) of 209±151 mm^2 (not significant).

In patients with normal plasma zinc concentrations (n=8), all zinc applied skin test responses were greater than zinc free ointment responses with a mean difference in area of 90±26 mm^2. Although the number of patients in this subgroup was small, the difference in skin test areas between zinc applied and zinc free sites was statistically significant (t=3.462, p<0.05).

Correlations between zinc associated skin test enhancement (change in area) and plasma zinc concentrations, zinc/copper ratios, serum albumin, and hemoglobin concentration were not significant for any of the groups.

DISCUSSION

This study demonstrates an augmenting effect of topical zinc on delayed type skin test reactivity without a correlation between degree of augmentation and plasma zinc levels. These results do not corroborate an earlier report on malnourished children (6), showing a relationship between low plasma zinc concentrations and topical zinc enhancement of skin tests. There may be several reasons for this difference: 1) topical zinc absorption into the skin may vary in different patients depending on age, disease, medication or other factors; 2) individuals may have varying thresholds for the effects of absorbed zinc on immune response; and 3) plasma zinc may not have accurately reflected patient zinc nutritional status in this study.

Although zinc absorption has been studied in animals (8) and burn patients (9), absorption in intact human skin has been seldom studied (10), especially in sites undergoing an active immunologic inflammatory response. Thus it is difficult to speculate on the effects of disease, medications, and other factors. Studies of transdermal zinc absorption in different kinds of dermatologic cellular reactions would be helpful in this regard. In our study a primary irritant reaction or a contact dermatitis (11) was excluded, with negative reactions at the saline sites applied with zinc sulfate ointment.

Plasma zinc levels, although useful in the assessment of zinc nutritional status, are not definitive indications of zinc deficiency. Some diseases such as lung cancer, tuberculosis, and alcoholic liver disease are known to be associated with depressed plasma zinc (12). However, such subjects with low plasma zinc concentrations are not necessarily zinc deficient, but may be experiencing a flux of zinc from plasma to liver, a phenomenon that occurs in a variety of diseases (12). Conversely, subjects with normal plasma zinc concentrations may have low tissue zinc stores. Although it is still possible that topical zinc augmentation of skin test reactivity relates to zinc nutritional status, plasma zinc concentrations as a sole index of zinc nutriture were not shown to correlate with zinc augmented skin test responses in this study.

The number of patients in this study does not allow for analyses of factors predisposing to topical zinc skin test enhancement. Because augmentation was seen in normals as well as patients, low plasma zinc is clearly not prerequisite. Since delayed type hypersensitivity reactions appear to be enhanced locally in the skin, zinc induced immunopotentiation may aid treatment of patients afflicted by chronic cutaneous infections by fungi, viruses, or mycobacteria. Host defense against these organisms is known to involve cell mediated immunity. Indeed, a beneficial effect of zinc on leprosy skin ulcers (13), and recurrent herpes simplex (14), has been observed. In addition the ability of local immunotherapy to cause regression of cutaneous neoplasia suggests augmentation of delayed hypersensitivity reactions may be helpful in cancer treatment (15). The results of our study strongly suggest that the clinical effects of topical zinc deserve further study.

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