

Zinc Salts Effects on Granulocyte Zinc Concentration and Chemotaxis in Acne Patients

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To explore the mechanism by which zinc acts on cutaneous inflammatory lesions, we studied granulocyte zinc levels and in vitro polymorphonuclear leukocyte chemotaxis in 20 acne patients before and after 2 months of zinc therapy (200 mg/day zinc gluconate).

The zinc level was assayed by flame absorption spectrophotometry and chemotaxis was performed by agarose assay. After 2 months of treatment, a significant decrease in granulocyte zinc level associated with inhibition of chemotaxis ($r = 0.69$) was observed in 16 patients. This suggests that zinc anti-inflammatory action is related to inhibition of polymorphonuclear leukocyte chemotaxis induced by a decreased granulocyte zinc level.

(Accepted December 9, 1991.)

Acta Derm Venereol (Stockh) 1992; 72: 250-252.

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Several studies (1-3) have demonstrated the efficiency of zinc salts especially as an alternative to tetracycline therapy (4) in the treatment of inflammatory acne lesions. However the mechanism involved is still unclear. Our earlier work has shown that granulocyte zinc concentration is increased in acne patients and that clinical response to zinc therapy is directly correlated with a decrease in granulocyte zinc concentration during treatment (5). This suggests that there is a relation between zinc anti-inflammatory action and inhibition of polymorphonuclear leukocyte (PMN) functions (chemotaxis, bactericidal effects) as a result of lower granulocyte zinc concentration. The present study investigates PMN chemotaxis relative to granulocyte zinc concentration in 20 patients before and after 2 months of zinc therapy.

MATERIAL AND METHODS

Patients

Twenty acne patients, (9 males, 11 females, 16-36 years) were studied between January and April 1990. All had grade II to IV acne (4 IV, 13 III, 3 II) according to the classification of Pillsbury et al. (6). None had used tetracyclines or zinc during the 3 preceding months. Except for their acne, all were in good health, without infectious disease, and received no other treatment during the study.

Examination procedure

Each patient was examined before and after the treatment period. The various types of lesions except comedones were counted (2) and acne grade was evaluated. Most patients were also photographed. Each received zinc gluconate (Labcatol*) 200 mg/day during treatment. Venous blood samples before and after treatment were always drawn in the morning (9 to 10 a.m.) after at least 12 h of fasting. The study was performed between November and February, to avoid seasonal variations.

Granulocyte and plasma zinc concentrations

Care was taken to avoid contamination from materials and laboratory reagents. Venous blood was collected in acid-washed polystyrene tubes containing preservative-free heparin (5U/ml blood). Granulocytes were isolated by Dextran and Ficoll Hypaque gradient (Pharmacia), which was controlled to be without zinc traces. Cells were washed 3 times in Hanks balanced salt solution, and contaminating erythrocytes were removed by treatment with hypotonic saline solution. Counts performed on an aliquot of the suspension showed that more than 95% of the white cells were neutrophils. Granulocyte and plasma zinc were assayed by flame absorption spectrophotometry in 35 healthy subjects [normal values respectively 6.3 ± 1 ng/106 cells (\pm SD), and 750 ± 150 mg/100 ml (\pm SD)].

Neutrophil chemotaxis under agarose

PMN chemotaxis was determined before and after the period of zinc therapy. Granulocytes were isolated by Dextran and Ficoll-Hypaque gradient, washed 3 times in Hanks balanced salt solution and resuspended in the appropriate medium at 5.0×10^7 PMNN/ml. Formyl-methionyl-leucyl-phenylalanine (FMLP, Sigma*) 10^{-7} M was used as attractant. Determination of chemotactic PMN migration was performed by agarose assay, as described by Repo H (7) using a medium consisting of 1% agarose and 10% fetal calf serum (FCS, Eurobio*) in Krebs Buffer (pH = 6.8). FCS was previously inactivated by 30-min heating at 56°C. This agarose medium (IBF*) in 4-ml volumes was placed in disposable tissue culture dishes (NUNC*) and allowed to solidify. Four groups of 3 wells each were then cut in the agarose gel using a 2.5 mm diameter punch. In two groups of wells, patient granulocytes (50×10^5 PMN) were placed in the middle well and FMLP (50 ml) and Krebs buffer (50 ml) in the other two wells. At the same time, control granulocytes from healthy subjects were placed in two other groups of wells. Krebs buffer was used to control spontaneous migration.

Dishes were incubated for 90 min at 37°C in a mixture of 5% CO₂ in air. Migration distance was determined in millimeters. The following ratio was used to estimate PMN response to FMLP:

$$\text{chemotaxis index (CI)} = \frac{\text{migration distance of patient PMN}}{\text{migration distance of healthy subject PMN}}$$

Normal chemotaxis value in healthy subject = 1 ± 0.02 .

Untreated control granulocytes for determination of chemotactic PMN migration before and after treatment were obtained from healthy young adult subjects without acne and not receiving zinc therapy.

Statistical evaluation

Results are shown as mean \pm SD. The Wilcoxon rank test was used for statistical comparisons of groups before and after treatment, and the Spearman rank correlation test for the association of granulocyte zinc level and chemotaxis index.

RESULTS

Granulocyte zinc level (Fig. 1)

Granulocyte zinc level was increased in 17 patients before treatment [mean (m) 15.33 ± 8.64 ng/106 cells for the 20 patients], and decreased in 17 patients after 2 months of zinc

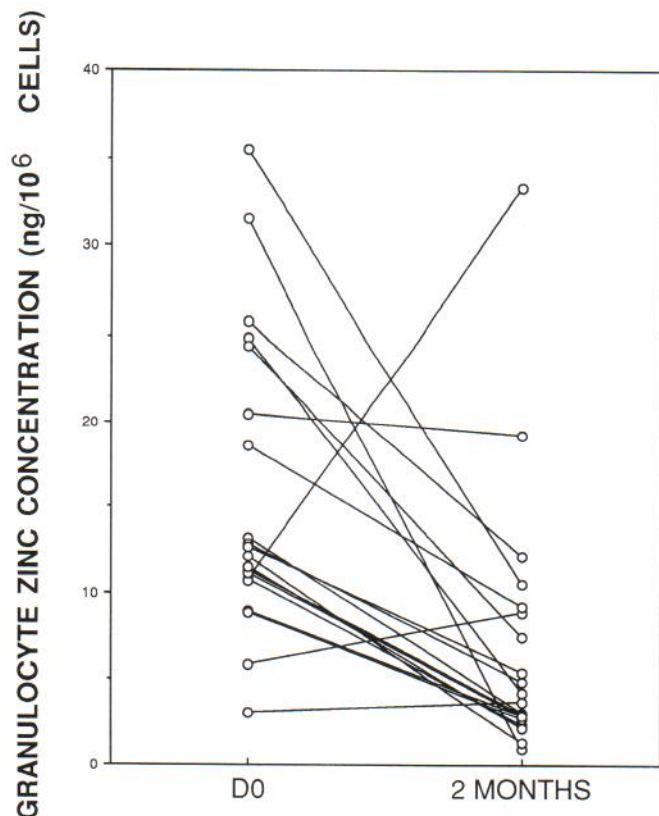


Fig. 1. Decrease ($p < 0.01$, Wilcoxon rank test) of granulocyte zinc concentration in 17 out of 20 acne patients after 2 months of zinc therapy.

therapy (mean 7.24 ± 7.73 ng/10⁶ cells for the 20 patients) ($p < 0.01$).

Plasma zinc level

Mean plasma zinc level for the 20 patients was normal before treatment (737 ± 170 ng/100 ml) and significantly increased (989 ± 275 ng/100 ml) after treatment. Individually, 4 patients had decreased levels after treatment.

The chemotaxis index (Fig. 2) was increased in 17 patients before treatment (mean for the 20 patients = 1.36 ± 0.38) and decreased in 16 after therapy (mean of the 20 patients = 1.01 ± 0.16) ($p < 0.01$).

The correlation (Fig. 3) was significant ($r = 0.69$) between decreased granulocyte zinc concentration and decreased chemotaxis index after zinc therapy.

Clinical course: before treatment, 3 patients had grade IV acne, 16 grade III and 1 grade II. After 2 months of treatment with zinc gluconate 200 mg/day, 16 showed clinical improvement, with a decreased inflammatory lesions (papules, pustules and nodules) and acne score (13 grade I, 3 grade II). 4 patients (3 male, 1 female) showed no improvement (13 grade I, 1 grade II) with persistence but no increase of papules, pustules or, in one case, nodules. No anti-inflammatory effect was noted for these 4 patients. Granulocyte zinc concentration remained high for 3 of these unimproved patients, plasma zinc level decreased in 1, and the chemotaxis index increased for all 4.

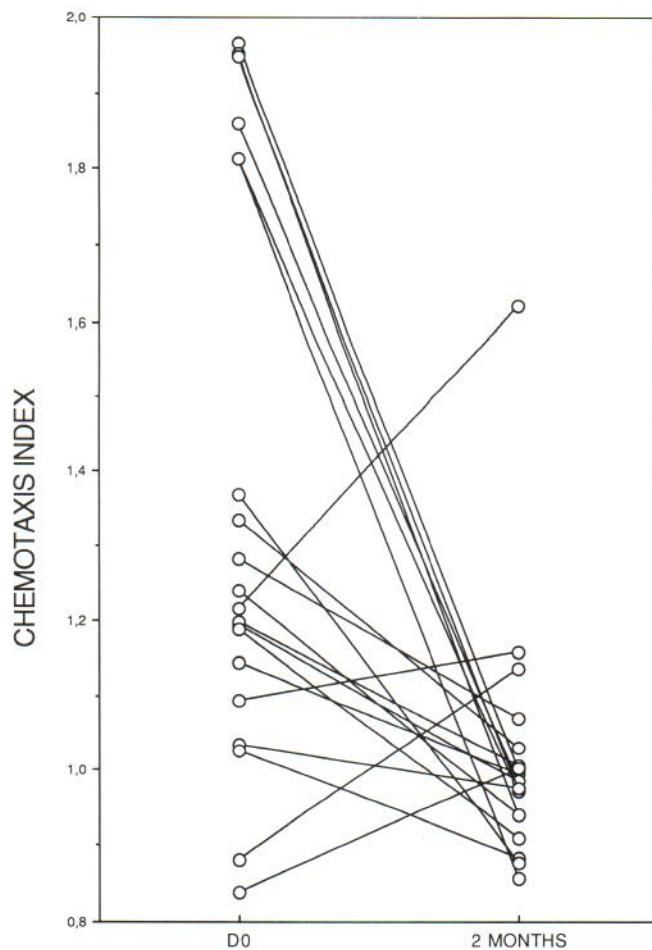


Fig. 2. Inhibition ($p < 0.01$, Wilcoxon rank test) of chemotaxis index in 16 out of 20 acne patients after 2 months of zinc therapy.

DISCUSSION

Granulocyte zinc level was increased in our grade II to IV acne patients before treatment. Inflammatory stress is known to cause increased zinc uptake in the liver (8), which suggests that granulocyte zinc uptake might also increase. In this context, high granulocyte zinc concentration has been found in inflammatory bowel disorders such as active Crohn's disease (9).

Before zinc therapy, 17 of our 20 acne patients had high zinc granulocyte levels associated with an increase in the chemotaxis index. After therapy, there was a parallel decrease in zinc concentration and chemotactic index in 16 patients, with a significant correlation ($r = 0.69$) between these two factors. These 16 patients all had pustules or inflammatory lesions before treatment which, after 2 months of treatment, decreased or disappeared, although retentional lesions were still present.

Increased chemotaxis and granulocyte zinc concentration before treatment was not related to the number of lesions or the intensity of the inflammation.

In 3 of the 4 patients without clinical improvement and with persistence of inflammatory lesions, the acceleration of chemotaxis and the rise in granulocyte zinc concentration after 2 months of therapy were associated with an increase in

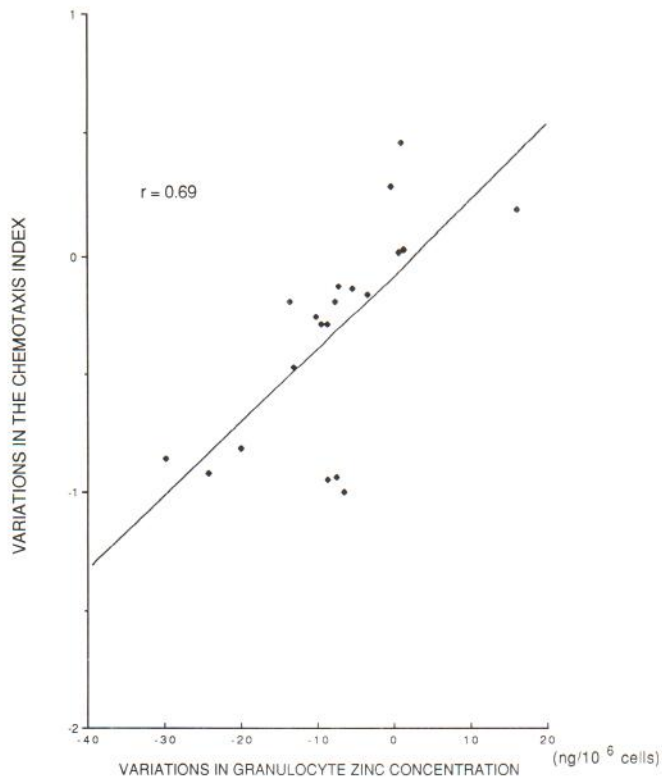


Fig. 3. Correlation ($r = 0.69$) between the decrease of granulocyte zinc concentration and inhibition of chemotaxis in acne patients treated with zinc therapy.

plasma zinc concentration. The clinical lesions of these patients were similar (not particularly severe acne) to those of patients with good results, and the treatment was well tolerated.

Our results suggest that the decrease in chemotaxis after zinc therapy was related to the decrease in granulocyte zinc concentration induced by zinc therapy which at the same time increased plasma zinc concentration. In the context of zinc deficiency, previous *in vitro* studies (10, 11, 12) have shown a relation between low zinc concentration in medium and enhanced PMN functions and between increased medium zinc content and inhibition of PMN functions (as obtained *in vivo* in our patients using zinc salts).

The mechanism by which increased medium zinc content produces an inhibition of granulocyte zinc functions is partly unknown. Our results suggest that the decrease in granulocyte zinc concentration involves mechanisms operating at the membrane as well as the intracellular level. Zinc may interact with carboxyl groups on plasma membrane, thereby causing changes in fluidity and membrane stability. Several plasma membrane enzymes are regulated by zinc (13), and zinc may compete with Ca^{++} which plays an important role in micro-

skeletal functions. *In vitro* the inhibitory effects of zinc on PMN functions are not immediate (incubation is required) and reversible (14). Inhibition of chemotaxis could thus have accounted for the clinical improvement and fewer inflammatory lesions noted in 16 patients in our study. However, the mechanism leading to a decrease in granulocyte zinc concentration under zinc therapy remains unclear.

ACKNOWLEDGEMENT

We thank Miss Arlette Bénardin for her excellent technical assistance.

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