Acta Derm Venereol (Stockh) 1986; 66: 305–310

The Effect of Oral Zinc Administration on Sebum Free Fatty Acids in Acne vulgaris

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Rebello T, Atherton DJ, Holden C. The effect of oral zinc administration on sebum free fatty acids in acne vulgaris. Acta Derm Venereol (Stockh) 1986; 66: 305–310.

Free fatty acids in sebum arise from lipolytic action of bacterial lipases. We have demonstrated an inhibitory effect of zinc on the lipase of the three *Propionibacterium* species found in human pilosebaceous follicles. We were also able to show a small corresponding fall in the free fatty acid content of skin surface lipid in vivo in acne patients treated with zinc, though this failed to reach statistical significance. *Key words: Free fatty acids; Bacterial lipase.* (Received November 11, 1985.)

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Oral zinc administration has been evaluated in the treatment of acne vulgaris in several centres (1–9). Unfortunately, the results of these trials have been conflicting, and it remains unclear whether zinc has any therapeutic role in this condition. We were nevertheless interested to investigate mechanisms by which such a therapeutic effect might be produced. We were aware that zinc has been shown to inhibit pancreatic lipase (10), and were therefore keen to determine whether zinc could inhibit the lipases of follicular bacterial species in vitro, as it has in the past been suggested that free fatty acids (FFA) in sebum may have a role in the pathogenesis of acne vulgaris (11, 12). We were also keen to establish whether such in vitro inhibition of follicular bacterial lipase would be reflected in a decrease in the proportion of FFA present in the skin surface lipid of zinc-treated subjects in vivo.

METHODS

Subjects

Eight individuals with moderate-to-severe acne vulgaris (7 male, 1 female, age range 18–24 years) received no treatment for at least 1 month prior to commencement of the investigation. Eight subjects without acne (7 male, 1 female, age range 22–24 years) acted as controls. All were instructed not to alter their dietary habits during the period of study. Both groups took capsules containing 220 mg of zinc sulphate heptahydrate (Zincomed, Medo-Chemicals, London, England) 3 times daily, 1 hour before meals, for a period of 1 month.

The following investigations were performed in all subjects immediately before and at the conclusion of the period of zinc administration.

Skin surface lipid sampling

The method of removal of skin surface lipids was as reported previously (13). Subjects reported in the morning having taken a bath the previous night. Casual sebum lipid was removed by solvent extraction (hexane : dietyl ether 1 : 1) from test sites of fixed area overlying the right and left scapulae. These test areas were protected from contact with clothing by means of polyethylene cups, and three hours later, the excreted sebum was collected. Total sebum was estimated gravimetrically. Sebum from the right and left scapular sites were processed separately.

Estimation of FFA

Total sebum was dissolved in chloroform, and free fatty acids were estimated as the copper-complex, by the method of Duncombe (14).

Plasma zinc

Venous blood samples were taken in the early morning following a 12-hour fast, and then stored at −20°C in special zinc-free polypropylene vials until analyzed. Plasma zinc was determined by atomic absorption spectroscopy (Instrumentation Laboratories, 751 spectrometer) (15).

Isolation of lipase from skin bacteria

Propionibacterium acnes (Strain P37), *Propionibacterium avidum* (Strain SP771), and *Propionibacterium granulosum* (Strain 208) (isolated from patients with acne vulgaris, and supplied by Dr K. Holland, Department of Microbiology, University of Leeds, England) were cultured in Brain Heart Infusion broth (Difco®) containing 1% glucose, under anaerobic conditions. Following the establishment of the stationary growth phase (approximately 1 week), the bacteria were harvested by centrifugation, and the supernatant (3 litres) was concentrated ten-fold by ultrafiltration. Ammonium sulphate was added until 60% saturated, and the pH adjusted to 6.0. After stirring for 1 hour at 4°C, the precipitate was centrifuged down, redissolved in distilled water (10.0 ml) and dialyzed 3 times against 0.005 mM dithiothreitol in 0.1 M sodium chloride. The dialyzed material was used for lipase assay, without further purification.

Lipase assay

Lipase activity was determined by the method of Nilsson-Ehle & Schotz (16), using tri-(9–10^{3}H) oleoylglycerol (Amersham, Bucks, England) as substrate. ^{3}H-labelled oleic acid liberated during the incubation was isolated by liquid-liquid partition system as described by Belfarge & Vaughan (17), and aliquots were assayed for radioactivity in a scintillation counter. Lipase from *P. acnes* was assayed at pH 5.0, and lipases from *P. avidum* and *P. granulosum* were assayed at pH 7.0. For the zinc inhibition studies, various concentrations of zinc sulphate (Sigma®) were pre-incubated at 37°C with the enzyme for 15 min, and then incubated with the substrate. Enzyme activity was expressed as μmoles of ^{3}H-labelled oleic acid liberated/ml enzyme/hour, and each assay was performed in triplicate.

RESULTS

Two subjects in the acne group (1 male and 1 female) did not complete the trial because of nausea induced by the zinc sulphate.

For statistical analysis, Student's *t*-test was used to compare pre- and post-treatment values in each subject (paired), and pre- or post-treatment values between the acne and control groups of subjects (unpaired).

Free fatty acid composition and sebum excretion rates from the right and left scapulary regions were similar. For convenience, the results were pooled.

Plasma zinc (Table I)

Fasting plasma zinc in the acne group was significantly lower than in the control group ($p<0.05$), but in only 1 case did it fall below normal (10.6 μmol/l; normal range 11.0–24.0 μmol/l). After 1 month of treatment, plasma zinc levels were significantly raised in both groups relative to pre-treatment values (control group: $p<0.005$; acne group: $p<0.02$). There was no significant difference in post-treatment values between the two groups.

Total sebum excretion rate (Table I)

Prior to treatment, the acne group had higher total sebum excretion rates than the control group ($p<0.05$). Zinc treatment did not reduce the total sebum excretion rate in either group.

Table I. *Effect of zinc treatment upon plasma zinc, and upon sebum excretion rate and composition of free fatty acids in patients with acne and controls*

All values are given as mean ±SEM

	Controls		Acne patients	
	Pre-treatment	Post-treatment	Pre-treatment	Post treatment
Plasma zinc, μmol/l (normal range: 11–24 μmol/l)	16.91±1.35	26.08±2.28	13.36[a]±0.86	22.28±2.77
	$p<0.005$		$p<0.02$	
Sebum excretion rate, μg/cm²/3 h	28.98±2.82	29.82±6.36	40.23[a]±4.42	39.01±5.65
	NS		NS	
Free fatty acid excretion rate, μg/cm²/3 h	2.59±0.47	2.49±0.37	4.21[a]±0.52	3.65±0.51
	NS		NS	
% of total lipid	9.14±1.68	9.48±1.57	11.6[b]±2.88	9.50±1.41
	NS		NS	

[a] Significantly different ($p<0.05$) from pre-treatment values in the control group.
[b] Not significantly different ($p>0.05$) from pre-treatment values in the control group.

Free fatty acid (Table I)

FFA is expressed both as the amount excreted per unit area over a 3-hour period (μg/cm²/3 h), and as a percentage of the total sebum lipid. Absolute sebum FFA was significantly higher before treatment in the acne group relative to the control group ($0.01>p<0.05$), but there was no difference in the relative FFA content of sebum. Zinc treatment did not significantly change free fatty acid composition in either group.

In vitro bacterial lipase studies (Table II)

Preliminary experiments showed the pH optimum for *P. acnes* lipase to be approximately pH 5.0. *P. granulosum* and *P. avidum* lipase showed pH optima around neutrality. These pHs were used for the zinc inhibition studies. *P. acnes* and *P. avidum* lipases showed approximately 80% inhibition at a zinc concentration of 2.0 mM/l, while lipase from *P. granulosum* was only inhibited by 36% at this concentration. At lower concentrations of zinc, *P. granulosum* lipase was not inhibited at all, whereas inhibition of *P. acnes* lipase was still detectable at 10 μM/l zinc sulphate.

DISCUSSION

It is unclear what role, if any, is played by zinc in the pathogenesis of acne vulgaris, or whether oral zinc supplements provide effective therapy. A major obstacle in solving these

Table II. *Inhibition of bacterial lipase in vitro by various concentrations of zinc sulphate*

Zinc sulphate Concentration (μmol/l)	% inhibition of lipase activity[a]		
	P. acnes	*P. avidum*	*P. granulosum*
2000	86±8.2	78±6.5	36±2.9
100	41±3.1	19±2.0	0
10	32±2.9	0	0

[a] Mean ± SEM, 3 determinations.

problems has been the difficulty in establishing whether patients with acne vulgaris are in fact zinc-deficient. A normal plasma zinc level does not preclude marked tissue zinc depletion (18), and unfortunately, no more reliable index of body zinc status has yet been established, though determinations of leucocyte (19) and skin (20) zinc levels are promising approaches currently under evaluation. Marginal zinc deficiency may be prevalent in Western communities (21–23), and would have its maximum effects at times of rapid body growth, and during pregnancy and lactation. The question logically arises whether acne vulgaris could, at least in some individuals, be a clinical manifestation of marginal tissue zinc deficiency, precipitated by increased zinc requirements at puberty. Although low serum or plasma zinc levels have been reported in a few patients with acne vulgaris (24), the majority will be found to have levels within the normal range (4). In our own acne patients, the plasma zinc levels were within the normal range, but were significantly lower than those of the control group of individuals without acne. The prevalence of minor degrees of zinc deficiency will be affected by a number of factors including age, and diet (21–25), and this may partly explain the inconsistent findings of those who have investigated the value of zinc treatment for acne vulgaris.

The mechanism underlying the relationship between acne vulgaris and zinc is the subject of speculation. Michaëlsson et al. have, for example, suggested that zinc is beneficial in acne as a direct result of its ability to increase the serum level of retinol binding protein (24). An alternative explanation concerns the role of zinc in essential fatty acid (EFA) metabolism. Zinc appears to be required for normal onward conversion of linoleic acid to gammalinoleic acid (26), and at least some of the effects of zinc deficiency may result from inhibition of this conversion (27). EFA deficiency induces hyperkeratosis in animal (28), and there is good evidence that the initial lesion in acne vulgaris is hyperkeratosis of the opening of the pilosebaceous follicle leading to its obstruction (29).

A further mechanism whereby zinc treatment might influence acne vulgaris is by inhibition of FFA production within the pilosebaceous follicle. Sebum FFA is released from triglyceride by the action of bacterial lipases (30, 31), particularly those derived from *Propionibacterium* species. Our results show that, in vitro, the lipases of the three major *Propionibacterium* species are inhibited by zinc. *P. acnes* is the most abundant of these three bacteria, and the inhibitory effect of zinc was most marked in the case of lipase from this species. Inhibition was detected at zinc levels of 10 μM, which is less than is present in normal plasma. Technical difficulties prevented us from determining the zinc content of sebum. Nevertheless it seems likely that body zinc status could influence *P. acnes* lipase activity in vivo. Zinc treatment of our patients increased their plasma zinc levels, and presumably would also have increased the level of zinc within the follicular microenvironment. Pre-treatment free fatty acid excretion rates in acne patients were significantly higher than in controls but this difference was abolished after zinc treatment. Although we were able to show both an absolute and a relative decrease of FFA in the surface lipids of our small group of zinc-treated acne patients, this failed to reach statistical significance and we are therefore unable for the present to confirm that FFA production within the pilosebaceous follicle is inhibited by zinc treatment. It is of course possible that statistical significance would have been achieved had the study group been larger. Nevertheless, surface lipid sampling probably provides a poor picture of changes in sebum composition deep within follicles. Little lipid emerges from obstructed follicles, in which lipolysis is almost complete (32), and short-chain FFA's may diffuse out of follicles without even reaching the surface.

We believe that further studies are needed to elucidate the relationship between zinc metabolism and acne vulgaris. Particular attention should be directed to the possibility that acne may in many patients reflect marginal zinc deficiency. Failure to identify such a

subgroup among acne patients may underlie the apparently conflicting results of published therapeutic trials of zinc in this disease.

ACKNOWLEDGEMENTS

T. Rebello was in receipt of a research grant from the Dunhill Trust. We are grateful to Medo-Chemicals, Ltd., and to Stiefel Laboratories for supplies of zinc sulphate capsules and for financial assistance. The plasma zinc determinations were performed by Dr P. J. Aggett at the Institute of Child Health, London, W.C., England.

REFERENCES

1. Burton JL, Goolamali SK. Zinc and sebum excretion. Lancet 1973; 1: 1448.
2. Michaëlsson G, Juhlin L, Vahlquist A. Effects of oral zinc and vitamin A in acne. Arch Dermatol 1977; 113: 31–36.
3. Michaëlsson G, Juhlin L, Vahlquist A. A double-bind study of the effect of zinc and tetracycline in acne vulgaris. Br J Dermatol 1977; 97: 561–566.
4. Weismann K, Wadskov S, Søndergaard J. Oral zinc sulphate therapy in acne vulgaris. Acta Derm Venereol (Stockh) 1977; 57: 357–360.
5. Hillström L, Petterson L, Helbe L, Kjellin A, Leczinsky LG, Nordwall L. Comparison of oral treatment with zinc sulphate and placebo in acne vulgaris. Br J Dermatol 1977; 97: 681–684.
6. Orris L, Shalita AR, Sibulkin D, London SJ, Gans EH. Oral zinc therapy of acne. Absorption and clinical effects. Arch Dermatol 1978; 114: 1018–1020.
7. Göransson K, Lidén S, Odsell L. Oral zinc in acne vulgaris: a clinical and methodological study. Acta Derm Venereol (Stockh) 1978; 58: 443–448.
8. Cunliffe WJ, Burke B, Doman B, Gould DJ. A double-blind trial of zinc sulphate/citrate complex and tetracycline in the treatment of acne vulgaris. Br J Dermatol 1979; 101: 321–325.
9. Verma KL, Saini AS, Dhamija SK. Oral zinc sulphate therapy in acne vulgaris: a double-bind trial. Acta Derm Venereol (Stockh) 1980; 60: 337–340.
10. Wills ED. The relation of metals and -SH groups to the activity of pancreatic lipase. Biochem Biophys Acta 1960; 40: 481–490.
11. Strauss JS, Pochi, PE. Intracutaneous injection of sebum and comedones. Arch Dermatol 1965; 92: 443–456.
12. Kligman AM, Katz AG. Pathogenesis of acne vulgaris: Comedogenic properties of human sebum in the external ear canal of the rabbit. Arch Dermatol 1968; 98: 53–57.
13. Rebello T, Hawk JLM. Skin surface glycerol levels in acne vulgaris. J Invest Dermatol 1978; 70: 352–354.
14. Duncombe WG. The colorimetric micro-determination of long-chain fatty acids. Biochem J 1963; 88: 7–10.
15. Meret S, Henkin RI. Simultaneous direct estimation by atomic absorption spectrophotometry of copper and zinc in serum, urine, and cerebrospinal fluid. Clin Chem 1971; 17: 369–373.
16. Nilsson-Ehle P, Schotz MC. A stable, radioactive substrate emulsion for assay of lipoprotein lipase. J Lipid Res 1976; 17: 536–651.
17. Belfarge P, Vaughan M. Simple liquid-liquid partition system for isolation of labeled oleic acid from moieties with glycerides. J Lipid Res 1969; 10: 341–344.
18. Garretts M, Molokhia M. Acrodermatitis enteropathica without hypozincaemia. J Pediatr 1977; 91: 492–494.
19. Keeling PNN, Jones RB, Hilton PJ, Thompson RPH. Reduced leucocyte zinc in liver disease. Gut 1980; 21: 561–564.
20. Michaëlsson G, Ljunghall K, Danielson BG. Zinc in epidermis and dermis in healthy subjects. Acta Derm Venereol (Stockh) 1980; 60: 295–299.
21. Sandstead HH. Zinc nutrition in the United States. Am J Clin Nutr 1973; 26: 1251–1260.
22. Hambidge KM, Walravens PA, Brown RM, Webster J, White S, Anthony M, Roth ML. Zinc nutrition of pre-school children in the Denver Head Start program. Am J Clin Nutr 1976; 29: 734–738.
23. Hambidge KM, Chavez MN, Brown RM, Walravens PA. Zinc supplementation of the low-income, pre-school children. In: Kirschgessner, M ed. Trace element metabolism in man and animals. Freising-Weihanstephan: Technische Universität München, 1978; vol. 3: 296–299.
24. Michaëlsson G, Vahlquist A, Juhlin L. Serum zinc and retinol binding protein in acne. Br J Dermatol 1977; 96: 283–286.

25. Oberleas D, Harland BF. Nutritional agents which affect metabolic zinc status. In: Brewer G J, Prasad A S eds. Zinc metabolism: Current aspects in health and disease. New York: Alan R. Liss, Inc., 1977: 11–24.
26. Horrobin DF, Cunnane SL. Interactions between zinc, essential fatty acids, and prostaglandins. Med Hypotheses 1980; 6: 277–296.
27. Cunnane SL, Horrobin DF. Parenteral linoleic and γ-linoleic acids ameliorate the gross effects of zinc deficiency. Proc Soc Exp Biol Med 1980; 164: 583–588.
28. Prottey C. Essential fatty acids and the skin. Br J Dermatol 1976; 94: 579–586.
29. Knutson DD. Ultrastructural observations in acne vulgaris: the normal sebaceous follicle and acne lesions. J Invest Dermatol 1974; 62: 288–307.
30. Marples RR, Downing DT, Kligman AM. Control of free fatty acids in human surface lipids by *Corynebacterium acnes*. J Invest Dermatol 1971; 56: 127–131.
31. Shalita AR. Genesis of free fatty acids. J Invest Dermatol 1974; 62: 332–335.
32. Nicholaides N, Ansari MN, Hwei L, Fu HC, Lindsay DE. Lipid composition of comedones compared with that of human skin surface in acne patients. J Invest Dermatol 1970; 65: 487–495.