Beneficial Effects of Oral Zinc Supplementation on the Immune Response of Old People

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Zinc is known to have beneficial effects on the immune response. In an attempt to modify age-associated immune dysfunction, supplemental zinc was administered to 15 subjects over 70 years of age (220 mg zinc sulfate twice daily for a month). As compared to 15 controls, matched for age and sex, there was a significant improvement in the following immune parameters in the treated group: (1) number of circulating T lymphocytes; (2) delayed cutaneous hypersensitivity reactions to purified protein derivative, Candidin and streptokinase-streptodornase; (3) immunoglobulin G (IgG) antibody response to tetanus vaccine.

Zinc treatment had no influence on the number of total circulating leukocytes or lymphocytes, or on the in vitro lymphocyte response to three mitogens: phytohemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM). The data suggest that the addition of zinc to the diet of old persons could be an effective and simple way to improve their immune function.

Aging is associated with a progressive alteration of immune competence affecting mainly but not exclusively T lymphocyte function. Immune dysfunction is thought to explain the increased incidence of autoimmune phenomena, amyloidosis and paraproteinemia in old people as well as their increased susceptibility to some infections [1].

Animal experiments have clearly demonstrated that selective zinc deficiency results in profound diminution in thymus size and function as well as in the T cell-dependent limb of the immune response. These defects can be completely corrected by zinc repletion [2,3]. Similar observations have been made in human subjects with acrodermatitis enteropathica [4,5], during total parenteral nutrition [6] and in protein-calorie malnutrition [7,8]. Available information based on serum zinc determinations suggests that old people have marginal zinc deficiency [9].

In the present study we have investigated the influence of oral zinc administration on the following immune parameters: number of circulating T lymphocytes, in vitro lymphocyte stimulation by T cell mitogens, delayed skin reactions to common antigens and IgG antibody response to tetanus vaccine.

MATERIALS AND METHODS

Participants and Experimental Schedule. The study was performed by comparing two groups, matched for age and sex, of institutionalized healthy people over 70 years old. The participants were assigned at random to either the control group or the treated group. Each subject was considered to be healthy on the basis of a clinical and biologic routine check-up. The only difference between the two groups of participants was the oral intake of 220 mg

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TABLE I Influence of Zinc Supplementation of Circulating Lymphocytes

Subjects	Bleeding Number*	Leukocytes†	Lymphocytes†	E-RFC‡
Treated (15)	I	7.700 ± 2.040	2.044 ± 663	57 ± 2.9
	II	6.800 ± 1.700	1.985 ± 584	67.5 ± 1.2§
Untreated (15)	I	6.900 ± 1.420	1.870 ± 691	60 ± 2.0
	II	7.400 ± 2.300	2.021 ± 493	58 ± 3.4

* Bleeding number: I = before zinc supplementation; II = after one month treatment.
† Number of cells/mm^3; mean ± SD.
‡ Expressed in percent; mean ± SEM.
§ $p < 0.05$, paired t test.

zinc sulfate after breakfast and dinner during one month. Each in vitro and in vivo test was performed simultaneously on the same number of subjects from each group and by the same laboratory personnel. Skin testing was performed by the same person ignorant of the group to which the subject was assigned. Tetanus vaccination was performed at the end of the treatment period by an intramuscular injection of 0.5 ml Tevax (RIT Lab.; Genval, Belgium). The immune response of the participants had been compared to that in young adults (<40 years) prior to and during the study. Old people had a reduced level of E rosette-forming cells (young adults: 65 ± 5 percent, mean ± standard deviation; participants: 58.6 ± 3.2 percent) and a lower response to skin tests (mean diameter of positive reactions was 65 percent of that measured in young subjects). Their lymphocyte response to PHA and Con A was reduced to 45 percent of the level observed in young adults.

Lymphocyte Studies. Lymphocytes were separated from 25 ml of heparinized blood by density-gradient centrifugation on Ficoll-Hypaque; the cells were then washed three times in Hanks' solution without calcium and magnesium (Gibco-Biocult.). The number of T cells was determined by their ability to form E rosettes according to the method of Jondal, Holme and Wigzell [10,11].

Lymphocyte cultures were performed as previously described [10,12] in RPMI 1640 (Flow Lab) containing 10 percent AB serum, 5 mM glutamine and antibiotics; the cultures were supplemented with purified PHA, 1 μg/ml (Wellcome Lab.); Con A, 5 μg/ml (Miles Lab.); or PWM 1/100 dilution (Gibco-Biocult.).

The lymphocyte response was measured by the uptake of tritiated thymidine which was added 18 hours before termination of the cultures.

Skin Tests. One tenth milliliter of the following solution was injected intradermally into the forearm: Candidin (Beecham; dilution 0.5 M), streptokinase-streptodornase (10 U/0.1 ml) and purified protein derivative (Statens Serum Inst., Denmark; 2 U/0.1 ml). The size of the indurated reaction was measured after 48 hours.

Antibody Response to Tetanus Toxin. IgG antibodies to tetanus toxin were measured by a solid-phase radioassay [13]. Polystyrene microtubes coated with purified tetanus toxin were incubated overnight at room temperature with 200 μl of a 1/50 dilution of test serum. The microtubes were then washed and further incubated for 6 hours with 200 μl of ^{125}I-labeled protein A from Staphylococcal aureus (Pharmacia, Sweden). After several washes, the tubes were counted and the bound radioactivity was taken as measure of IgG antitetanus toxin antibodies. Antibody titers were determined by reference to a standard curve obtained by serial twofold dilutions (1:50 to 1:3,200) of an hyperimmune serum. The blank was obtained by using a serum from a nonimmune young healthy subject; the same serum, diluted 1:40, was used for the serial dilutions of the standard serum. Titers were measured on blood samples taken before vaccination and three weeks later.

RESULTS

Mean age was 81 ± 5 years in the treated group and 79.6 ± 4.2 years in the controls. Sex ratios were comparable (six to nine males versus seven to eight females). There was no modification in the number of circulating leukocytes or lymphocytes after one month of zinc supplementation. However, the proportion of T lymphocytes significantly increased in the treated group ($p < 0.05$, paired t test) (**Table I**). This modification in the pool of circulating E rosette-forming cells was not accompanied by an effect of zinc treatment on the in vitro lymphocyte response to PHA, Con A or PWM (**Table II**). Oral zinc had a pronounced influence on the delayed skin responses to purified protein derivative, Candidin and streptokinase-streptodornase. As shown in **Table III** the frequency of positive responses increased from 60 percent to 84.5 percent in the treated group of old people whereas no modification was observed in the controls (69 percent versus 67 percent, chi square p

TABLE II Influence of Zinc Treatment on Lymphocyte Response to Mitogens

Subjects	Bleeding Number*	Mitogens		
		PHA	Con A	PWM
Treated	I	101 ± 16.4†	87 ± 12	63.5 ± 8.9
	II	121 ± 21.2	90 ± 14	75.4 ± 21.2
Untreated	I	134 ± 20.3	95 ± 24	56.4 ± 18.2
	II	151 ± 36	120 ± 32.4	62.1 ± 7.9

* See Table I.
† cpm × 10^{-3}; mean ± SEM.

<0.001). Furthermore the magnitude of the response was significantly increased after one month's treatment (p <0.001, paired t test). The IgG antibody response to tetanus toxoid vaccination was much greater in treated than in untreated subjects as shown both by a higher proportion of responders and by higher antibody titers among them (**Figure 1**). Geometric mean titer rose from 7 to 65 arbitrary units (AU) in the treated group and from 10 to 18 AU in the untreated group (p <0.001 paired Wilcoxon's test).

TABLE III Influence of Zinc on Delayed Hypersensitivity Skin Reactions

Subjects	Before Treatment	After 1 Month Treatment
	A. Number of Positive Tests	
Treated	27/45*	38/45
Untreated	31/45	30/45†
	B. Mean Diameter of Positive Reactions	
Treated	8.7 ± 1.4‡	15.4 ± 2.3§
Untreated	7.5 ± 1.0	8.9 ± 1.8

* Ratio between positive response and number of tests.
† $p < 0.01$ (Chi square).
‡ Expressed in mm; for each reaction two diameters were measured; mean value ± SEM.
§ $p < 0.001$ (paired t test).

COMMENTS

Attempts to improve the immune competence of aging man by means of pharmacologic agents are still poorly documented, and conflicting reports have been published, for example on the influence of levamisole [12,14-16].

The present results clearly show that in old people the oral administration of zinc significantly increases the antibody response to tetanus toxoid and ameliorates two classic parameters of cell-mediated immunity: the number of circulating T lymphocytes and the cutaneous delayed hypersensitivity reaction.

At a dose of 220 mg twice daily, zinc sulfate was well tolerated and only minor side effects were recorded. These were noted in five participants and consisted of transitory episodes of nausea and mild diarrhea.

The data do not indicate whether the beneficial influence of zinc is secondary to the correction of a latent zinc deficiency or to "immunostimulating" properties. Determination of serum zinc concentration was not available during the present study. Furthermore, current information indicates that the measurement of serum zinc concentration is not a valid reflection of zinc status [17,18]. The possibility of an immunostimulating effect of zinc supplementation, independent of zinc depletion, is supported by several observations. Firstly, it has been observed that in healthy young adults the oral administration of zinc increases in vitro lymphocyte response to mitogens [19]. Secondly, oral zinc augments the circulating pool of a T lymphocyte subpopulation in patients with acute lymphoblastic leukemia [20]. Finally, the addition of zinc to the drinking water of mice is associated with a time- and dose-dependent increase in mitogen stimulation of their spleen lymphocytes in vitro [21].

The present observation of an increase in the number of circulating T lymphocytes after zinc treatment might be explained either by a direct effect of zinc ion on the lymphocyte membrane or by a stimulation of thymus endocrine function.

The first possibility seems less likely, because a direct effect of zinc on cell membranes in vitro has generally been observed with concentrations one to two orders of magnitude higher than those obtained in vivo after the oral administration of zinc [19,22]. Nevertheless, this hypothesis cannot be formally excluded as it is known that the administration of zinc to patients with sickle cell anemia can inhibit the deformation of erythrocyte membranes characteristic of the disease [23]. The alternative explanation of an effect of zinc on thymic epithelial cells, leading to an improvement of their secretory activity seems more attractive. Indeed, during aging there is a pronounced but incomplete atrophy of the thymus accompanied by a parallel reduction in the level of thymic humoral factors [24,25]. These factors are known to control the expression of sheep red blood cell receptors on T lymphocytes [26,27]. The administration of zinc to experimental animals and to patients with hypogammaglobulinemia may lead to an increase in the serum level of thymic hormones [28,29]. This possibility could now be tested by measuring one or more of these thymic factors in the serums of treated and untreated subjects.

Further investigations are warranted to explain the mechanisms underlying the favorable influence of zinc

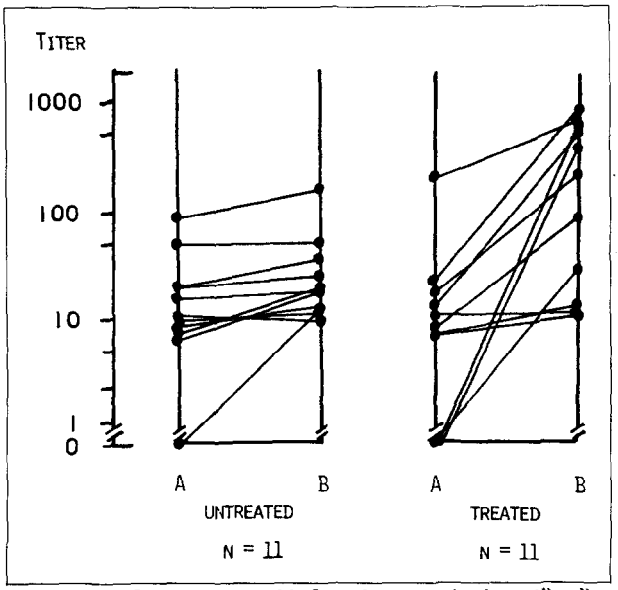

Figure 1. Comparison of IgG antitetanos toxin antibodies prior to (A) and three weeks after (B) vaccination in treated and untreated subjects. Titer is expressed as log of arbitrary units (see "Materials and Methods").

supplementation on the delayed skin reactions and on the antibody response to tetanus toxoid. These might be secondary to improvement in T lymphocyte function. Both phenomena are T cell-dependent, and zinc increases the number of circulating T lymphocytes. This interpretation is apparently contradicted by the absence of an effect of zinc treatment on the in vitro lymphocyte response to T cell mitogens. However, it must be noted that the ability of T cells to proliferate is largely independent of their function as mediators of delayed hypersensitivity reactions by secreting lymphokines or as helpers in the differentiation of B lymphocytes into antibody secreting cells [30,31].

The aforementioned effects of zinc could also be secondary to a modification of monocytes function or distribution. An increased activity of mononuclear phagocytes has been described in experimental animals during aging, and it has been related to their reduced antibody response to small doses of antigens [32,33]. The influence of age on the circulation and migration of monocytes is not known.

Therefore, although the underlying mechanisms are not yet clear, the present data justify a prospective study of the long-term effects of dietary zinc supplementation in old people. This regimen is unexpensive, nontoxic and could significantly improve immune function.

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