Eur J Cancer Clin Oncol, Vol. 25, No. 6, pp. 973–976, 1989.
Printed in Great Britain

0277-5379/89$3.00 + 0.00

T-Lymphocyte Dysfunction in the Elderly Associated with Zinc Deficiency and Subnormal Nucleoside Phosphorylase Activity: Effect of Zinc Supplementation

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Abstract—*The present study was conducted in an attempt to explore a possible mechanism for zinc-deficiency-induced T-lymphocyte dysfunction in the elderly. Eight elderly subjects aged* 65–78 *years served in this study. All subjects were anergic, zinc-deficient and had low activity of erythrocyte nucleoside phosphorylase. Baseline values for zinc status and immunological indices were established after which subject were treated with zinc acetate* (60 mg *elemental zinc per day given orally) for a period of* 4½ *months. Zinc supplementation resulted in a significant increase in the concentrations of zinc in plasma* ($P < 0.001$), *erythrocytes* ($P < 0.05$), *lymphocytes* ($P < 0.001$) *and neutrophils* ($P < 0.005$). *The activity of nucleoside phosphorylase in erythrocytes increased significantly* ($P < 0.001$) *as a result of zinc treatment. This was associated with significant improvement in the delayed cutaneous hypersensitivity reactions.*

INTRODUCTION

NUCLEOSIDE PHOSPHORYLASE (NPase), an enzyme essential for T-lymphocyte and B-lymphocyte functions, catalyzes the phosphorylysis of guanosine, inosine, deoxyguanosine and deoxyinosine. Patients with NPase deficiency accumulate large amounts of all four substrates in their urine, have low serum uric acid and excrete diminished amounts of uric acid [1]. *In vitro* enzymatic and metabolic studies have supported the hypothesis that the accumulation of the substrate of deoxyguanosine triphosphate (d-GTP) accounts for the T-cell specific effect of NPase deficiency (see discussion) such as delayed cutaneous hypersensitivity (DCH) reactions.

The necessity for zinc (Zn) for normal immune response has been well established [2–5]. Zinc deficiency in the experimental animal model has been associated with impaired cell-mediated immunity, poor growth of lymphoid organs and thymic atrophy [2]. Moreover, T-lymphocyte-mediated functions such as DCH reactions and T-helper and cytotoxic T-killer activities are adversely affected in the Zn-deficient state in animals [2, 3] and in human subjects [4, 5].

The elderly population exhibits clinical and laboratory evidence of immunologic dysfunction which is characterized by an increase in the incidence and severity of infections and impaired cellular and humoral immunity [6–8]. Beisel [9] reviewed increasing evidence that trace elements, particularly zinc, may affect the immune response in the elderly. Furthermore, Sanstead *et al.* [10] indicated that the elderly population is one of the groups which appears to have a high incidence of mild Zn deficiency which may account for the high incidence of immunological abnormalities in this population.

Therefore, the present study was conducted to test the effect of Zn supplementation on anergic, Zn-deficient elderly subjects with a low activity of erythrocyte NPase in an attempt to explore a possible mechanism for the Zn-deficiency induced T-lymphocyte dysfunction.

MATERIALS AND METHODS

Eight relatively healthy non-institutionalized elderly subjects participated in this study. They were all males aged 65–78 years, of lower socioeconomic status and able to sign a consent form. These subjects were selected among 50 elderly who volunteered to participate in another investigation in

Accepted 6 February 1989.
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which dietary assessment and nutritional status including immunological indices were studied. The selection of the eight subjects for this study was based on their low status of zinc, subnormal DCH reactions and low activity of NPase in erythrocytes as compared to 13 age and sex matched subjects who served as controls.

At the initiation of this study, baseline values were established for zinc concentration in plasma, erythrocytes, lymphocytes and neutrophils, activity of NPase in erythrocytes, anergy tests and routinc clinical tests. Each of these measurements was done twice within 2 weeks prior to the study and the means were considered as baseline values. Following the initiation of baseline values each subject was supplemented with 60 mg of elemental Zn (Zn acetate in capsules) daily for a period of 4½ months. Daily Zn supplement was given in two doses (30 mg each) 1 h before or after a meal. At the end of the supplementation period, the same parametric measurements of Zn status and immunological indices were conducted on both the Zn supplemented group and the control group, and compared with those of the baseline values using a paired *t*-test.

Zinc levels were measured in plasma, erythrocytes, lymphocytes and neutrophils by flameless atomic absorption spectrophotometry according to a technique we developed previously [11]. The activity of nucleoside phosphorylase was determined in erythrocytes by the technique described by Kalckar [12]. For the anergy test, each subject received a battery of four skin tests as described by Sokal [13]. The antigens used included purified protein derivative (250 u), streptokinase–streptodornase (400 u/100 u), mumps and *Candida albicans* (100 u). Each antigen was injected intradermally in a volume of 0.1 ml. All tests were read at 48 h. An area of induration of 5 mm or more was recorded as a positive reaction.

RESULTS

Table 1 shows the parametic measurements of zinc status and the immunological indices in the eight elderly subjects before and after 4½ months of zinc supplementation. Significant increases in the concentrations of zinc in plasma ($P < 0.001$), erythrocytes ($P < 0.05$), lymphocytes ($P < 0.001$) and neutrophils ($P > 0.005$) were observed as a result of zinc supplementation. The total lymphocyte count did not change significantly. The number of positive skin tests increased from 17/32 (positive response, 53%) to 25/32 (positive response, 78%). The mean diameter of positive reactions increased significantly ($P < 0.001$). As shown in Table 1, the activity of nucleoside phosphorylase in erythrocytes increased significantly ($P < 0.001$) in response to zinc supplementation.

DISCUSSION

The main thrust of the present investigation was to study the effect of Zn supplementation, in zinc-deficient elderly subjects, on DCH reactions and on the activity of nucleoside phosphorylase in an attempt to elucidate the mechanism of Zn-deficiency-induced T-lymphocyte dysfunction. Therefore, low zinc status, subnormal activity of nucleoside phosphorylase and abnormal DCH reactivity were used as criteria for the selection of the volunteers.

As shown in Table 1, all subjects were indeed Zn-deficient as indicated by the concentrations of Zn in plasma, red blood cells, lymphocytes and neutrophils. Values of Zn as shown in this study are lower as compared to those reported by others [14, 15]. This may be due to the differences in age and/or laboratory methodology. As expected, Zn supplementation for a period of 4½ months resulted in a significant increase in the concentrations of Zn in these tissues.

Although criticized as being a nondefinitive screening procedure, the DCH reaction test is the most important method for clinically evaluating cellular immune response [16]. Our results show that Zn supplementation improved anergy in elderly subjects who were previously Zn deficient. Ballester and Prasad [15] reported similar finding in three Zn-deficient sickle cell anemia patients who were supplemented with zinc for a period of 6 months.

Dachateau *et al.* [17] showed that pharmacological dosages of Zn (100 mg elemental Zn per day) given to elderly subjects for a period of 4 weeks resulted in improvement in DCH reactions, number of circulating T-lymphocytes, and IgG antibody responses to tetanus vaccine. However, Zn status prior to or after Zn supplementation was not assessed. Recently Bodgen *et al.* [14] reported the association between DCH reactivity and plasma Zn level. They suggested a systemic role for Zn in DCH reactions by showing that only a small difference (10 μg/dl) in plasma zinc may have an effect.

The relation of zinc to the integrity and function of the enzyme NPase, essential for T-lymphocyte and B-lymphocyte functions, has not been well defined. Cohen *et al.* [18] suggested d-GTP as a toxic metabolite in immune deficiency associated with purine NPase deficiency. Since it has been well established that Zn-deficient elderly subjects show a number of immunological abnormalities involving T-lymphocyte function and provided the fact that T-cell function is known to be affected adversely in a genetic disorder associated with nucleoside phosphorylase [1, 18, 19], we found it reasonable to investigate the effect of Zn supplementation on the activity of this enzyme in elderly subjects. Our results in an experimental animal model [20]

Table 1. Parametric measurements of zinc status and immunological indices in the elderly (mean ± S.D.)

	Baseline (before treatment)	After $4\frac{1}{2}$ months of treatment	Significance (P value)*	Controls†
Zinc concentration in:				
Plasma (μg/dl)	75 ± 15	115 ± 19	<0.001	98 ± 12
Erythrocytes(μg/gHb)	36 ± 9	43 ± 11	<0.05	45 ± 8
Lymphocytes(μg/10^{10} cells)	35 ± 10	51 ± 11	<0.001	49 ± 13
Neutrophils (μg/10^{10} cells)	48 ± 12	67 ± 9	<0.005	59 ± 10
Immunological indices:				
Total lymphocyte count (cells/mm^3)	1859 ± 420	1975 ± 285	N.S.	2011 ± 285
Number of positive skin tests‡	17/32 (53%)	25/32 (78%)	—	44/52 (85%)
Mean diameter of positive reactions (mm)	7.8 ± 2.1	14.1 ± 3.9	<0.001	16.6 ± 4.3
Erythrocyte NPase activity (OD/h/mg Hb)§	7.7 ± 2.1	13.6 ± 4.0	<0.001	16.5 ± 7

*Baseline values compared to those after $4\frac{1}{2}$ months of zinc treatment using paired t-test.
†Laboratory normal values for 13 age and sex matched healthy subjects.
‡Ratio between positive response and number of tests. Number in parentheses indicates the percentage of positive response.
§The change in optical density per hour per milligram hemoglobin.

showed a significant decrease in the activity of NPase, in liver and erythrocytes, in Zn-deficient as compared to pair-fed or ad lib-fed rats. The present study clearly demonstrates the increase in NPase activity in response to Zn supplementation. This effect was associated with improved DCH reactivity in the elderly subjects. Pilz *et al.* [21] provided evidence for the structural involvement of Zn ions in NPase associated with human lymphoblasts.

Two possible mechanisms may explain our present findings with regard to the effect of Zn supplementation in correcting the T-lymphocyte dysfunction manifested by abnormal DCH reactivity. The first mechanism involves the stimulation of NPase by Zn as demonstrated in the present study. The second possible mechanism involves the effect of Zn on DNA synthesis: Zn depletion in *in vitro* cultures significantly inpairs the ability of lymphocytes to synthesize DNA [22] and to produce antigen stimulated lymphokines [23], both essential steps in the process of DCH reactions. Thus zinc supplementation may reserve this effect. The present study and our previous results [20] in the experimental animal model provide support to the first mechanism in that zinc supplementation induces the activity of the enzyme, NPase, essential for normal T-lymphocyte function assessed by DCH reactivity test. Moreover, the early finding of lymphocyte depletion of NPase in the elderly [24] may be linked with our present observation of Zn-responsive immunoregulation of lymphocyte function in the aged.

Acknowledgements—The author acknowledges the technical assistance of M.T. Cossack, M.D. and S. Kassim, B.Sc.

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