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EFFECTS OF ZINC ON IMMUNE RESPONSE AND ASCORBIC ACID STATUS IN ALCOHOLICS

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The possible effect of zinc as a detoxicant and an immunostimulant has been evaluated by supplementing chronic alcoholics without overt liver disease with oral zinc sulphate. Satisfactory changes were observed after the supplementation and many of the altered features of the alcoholics showed tendency towards normalcy. After the therapy there was a reduction in serum γ-glutamyl transpeptidase activity, increase in the percentage of serum albumin, rosette forming cells, blastogenesis and DNA synthesis in proliferating T cells. On the other hand there was decrease in the level of serum immunoglobulin and both plasma and leucocyte dehydroascorbic acid. It seems that both blastogenesis and the increased rate of DNA synthesis in alcoholics after zinc therapy could be a direct effect of zinc or its action may be mediated by ascorbate /dehydroascorbate system through its antioxidant property.

Key words: Alcoholism, Hepatotoxicity, Immune Status, Zinc Therapy, Ascorbate /Dehydroascorbate System.

INTRODUCTION

There are several reports about the hepatotoxic effect of alcohol and immunological alterations in alcoholics with severe liver damage (1-5). Recently, while studying the immune status of chronic alcoholics at a pre-clinical stage in our laboratory, we have observed increased level of γ-glutamyl transpeptidase (GGT) activity, decreased percentage of serum albumin, increased serum immunoglobulin level, increased T cell percentage and decreased B cells in peripheral circulation, decreased percentage of rosette forming cells, decreased blastogenesis, increased rate of DNA synthesis in non activated cells and increased level dehydroascorbic acid in the blood. We suggested that the hepato cellular damage produced due to alcohol abuse possibly sensitize T cells leading to further aggravation of cirrhosis problems (6).

In another study it was observed that supplementation of large dose of ascorbic acid could restore some of the altered features of the alcoholic subjects (7). Since a moderate zinc deficiency has been observed in alcoholic cirrhosis patients (8) and recently it has been shown that zinc is essential for cell mediated immune function (9,10), we thought of trying out zinc therapy on a group of chronic subjects.

It has been observed by some workers that zinc has a protective effect against the hepatotoxicity of bromobenzene and acetaminophen (11). Several investigators (12-17) have shown that T helper cells, T suppressor cells, natural killer cells and antibody formation are dependent on, in addition to other factors, the status of zinc although the mechanism of the effect is not understood. Mild zinc deficiency has been reported to be associated with a reduction in the ability to produce IL-2 (18). It has been observed that zinc deficiency in adult mice caused significant reduction in the total number of splenic lymphocytes (19). Furthermore, Bumb and coworkers have evaluated the possible effect of zinc as an immunostimulant. They supplemented some multibacillary leprosy patients with oral zinc sulphate and observed satisfactory changes (20). A significant increase in DNA synthesis in proliferating lymphocytes has also been reported in children with Down's syndrome after oral administration of zinc sulphate

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(21). An impaired cell-mediated and humoral immunity has been observed in mild human zinc deficiency (22).

Thus a systematic study may be launched to test the efficacy of zinc therapy for alcoholics, especially to boost the immunity. The objective of the present investigation is to observe whether oral zinc supplementation has any protective effect on the damaged liver and on the altered immunological parameters associated with alcohol abuse in chronic alcoholics without apparent liver disease.

MATERIALS AND METHODS

Sixteen male habitual drinkers of age group 30-50 years with same socio-economic background and with a history of 5-15 years of alcohol abuse were chosen for the study. All the subjects were male and socio-economically they were classified as middle class, not very poor neither rich. Usually they were the only earning member of the family of 4-5 members and spent a minimum of 5-10% of their earning in alcohol purchase. No discrimination could be made on the nature and content of the hard drink. Sixteen normal controls (healthy, never touched alcohol) of the same age group and same socio-economic background were studied and normal parameters were obtained which were compared with that of alcoholics. Increased γ-glutamyl transpeptidase with normal AST/ALP was taken as a criteria for damaged hepatic function. The experimental group never complained about any specific disorders and were not treated for any hepatic condition. As such they could be classified under the heading of K70. In fact wherever we found any complication is associated, we did not include that subject in our study.

After the initial tests the subjects were treated orally with zinc sulphate at a dose of 660 mg per day in three divided doses for one month after which the different estimation were made according to standard protocols as outlined earlier (8). It may be pointed out al this stage that 220 mg of $ZnSO_4$ (equivalent to 50 mg of elemental Zn) thrice daily after meals for 32 days has been found to increase the rate of healing of granulating wounds with no evidence of toxicity (23-25). Patients continued drinking during the whole period of zinc supplementation. Each patient was informed about the importance of the present work and they voluntarily agreed to participate with a written consent.

Quantitation of GGT activity in serum was carried out by Szasz method (26). Serum total protein was determined by the Biuret method and serum albumin by the dye binding method (27). Serum was fractionated for different types of proteins by standard electrophoretic method (27). The percent of E. rosettes forming cells was determined in reference to total lymphocytes by the E-rosette technique (28, 29). T and B cell ratio of peripheral blood was obtained by nylon wool fibre column separation technique (30), Blastogenesis was measured by activating T Iymphocytes with phytohemagglutinin (PHA) added in tubes containing 1×10^6 cells in 1 ml culture medium. This suspension (0.2 ml) containing 2×10^5 cells were then added to each well of culture plate. The plates were then incubated at 37°C in a humidified atmosphere of 7.5% CO_2 in air for 48 hours. Blast cells were counted by haemocytometer in presence of trypan blue under the microscope fitted with anocculometer in presence of trypan blue under the microscope fitted with an occulometer. DNA synthesis of the proliferating T cells was measured by the degree of incorporation of ^{3}H-thymidine (TdR). Twelve hours prior to the time of harvesting 0.5 μCi of ^{3}H-TdR was added per well of culture plate containing 2×10^5 cells. At the end of incubation, after proper washes precipitation of DNA on nitrocellulose filter was made by 10% TCA and the individual filter discs were counted in Scintillation cocktail in Beckman LS 1800 Scintillation Counter (6). Plasma ascorbic acid and dehydroascorbic acid level was determined by 2,6-dichlorophenol indophenol titration. The 2,4-dinitrophenyl hydrazone method was utilized for the determination of leucocyte ascorbic acid and dehydroascorbic acid level (31). The Students t Test was used to determine statistical significance (32). A p value of less than 0.05 was considered significant.

RESULTS

There was a reduction in GGT activity in the alcoholics selected for zinc supplementation from a

mean value of 49.5 IU/I to 35.8 IU/I after the supplementation (Table 1). Zinc supplementation could restore the serum albumin level towards normalcy from a mean value of 3.9 g/dl to 4.3 g/dl (Table 1). Electrophoretic analysis of serum proteins also showed an increase, in the percentage of albumin after the therapy. Besides this there was an insignificant decrease in the percentage of both β and γ globulin as well as the values of α_1 and α_2 globulins (Table 1) after the supplementation. The percentage of B and T cells in the peripheral circulation remained almost the same after the therapy but there was a significant increase in the percentage of rosette forming cells from a mean value of 45.2% to 53.5% (Table 1). Blast formation with both 5 μg and 10 μg dose of PHA increased significantly after the therapy (Table 1). The increase is more significant with the 5 μg dose. Zinc supplementation caused a significant increase in the incorporation of ^{3}H-TdR both PHA treated and untreated lymphocytes. Interestingly, the incorporation of ^{3}H-TdR by the PHA treated lymphocytes was much higher than the untreated cells (Fig. 1). Although there was no significant change in the level of ascorbic acid, the level of hydroascorbic acid decreased to a good extent both in plasma and leucocyte (Table 1) in the alcoholics receiving zinc supplementation.

DISCUSSION

The restoration of serum GGT activity and serum albumin level towards normalcy after the supplementation suggest that zinc, as well as ascorbic acid (7), has a protective effect against alcohol induced hepatotoxicity. This could be due to the enhanced activity of the enzyme alcohol dehydrogenase. The ethanol metabolizing enzyme alcohol dehydrogenase is a zinc metaloenzyme (33) and rate limiting. The enzyme converts ethanol to acetaldehyde. The enzyme acetaldehyde dehydrogenase rapidly and irreversibly metabolizes acetaldehyde to acetate. With zinc supplementation there is a possibility of enhanced ethanol dehydrogenase activity, which may fasten the elimination of alcohol resulting in lower

Table 1. Changes in serum GGT activity, protein level, immunological parameters and plasma & leucocyte ascorbic acid and dehydroascorbic acid levels of before and after zinc therapy. GGT, γ-glutamyl transpeptidase; AA, ascorbic acid; DAA, dehydro-ascorbic acid

PARAMETERS	NORMAL (MEAN + SE)	ALCOHOLICS BEFORE ZINC THERAPY (MEAN + SE)	ALCOHOLICS AFTER ZINC THERAPY (MEAN + SE)	SIGNIFICANCE OF DIFERENCE (*p* VALUES)
Total serum protein (g / dl)	7.9 + 0.5	8.2 + 0.3	7.9 + 0.1	
Serum albumin (g / dl)	4.5 + 0.14	3.9 + 0.3	4.3 + 0.1	
GGT (IU / l)	11 + 1	49.5 + 4.1	35.8 + 2.1	< 0.01
Serum albumin (%)	58 + 0.9	49.6 + 1.4	55.1 + 1.2	< 0.01
α_1 globulin (%)	4.4 + 0.5	5.6 + 0.4	4.4 + 0.3	
α_2 globulin (%)	8.5 + 0.7	8.9 + 1.1	7.5 + 0.8	
β globulm (%)	10.5 + 0.3	14.6 + 1	13.3 + 1.1	
γ globulin (%)	18 + 0.9	21.3 + 0.9	19.5 + 0.7	
B cells (%)	38.8 + 1.3	28.7 + 3.2	28.3 + 2.7	
T cells (%)	61.2 + 1.3	71.3 + 3.2	71.7 + 2.7	
Rosette cells (%)	57 + 0.7	45.2 + 2.7	53.5 + 1.8	< 0.01
Blast formation with				
5 μg/ml PHA (%)	26.3 + 3.3	30.5 + 2.6	41 + 1.9	< 0.005
10 μg/ml PHA (%)	55.2 + 2	44 + 3.9	53.6 + 1.2	< 0.01
Plasma AA (mg / dl)	1 + 0.04	0.8 + 0.17	0.9 + 0.06	
Plasma DAA (mg / dl)	0.07 + 0.02	0.3 + 0.07	0.05 + 0.02	< 0.01
Leucocyte AA (μg / 10^8)	26 + 1.2	24 + 1	19 + 1	
Leucocyte DAA (μg / 10^8)	0	28 + 1	5 + 1	< 0.005

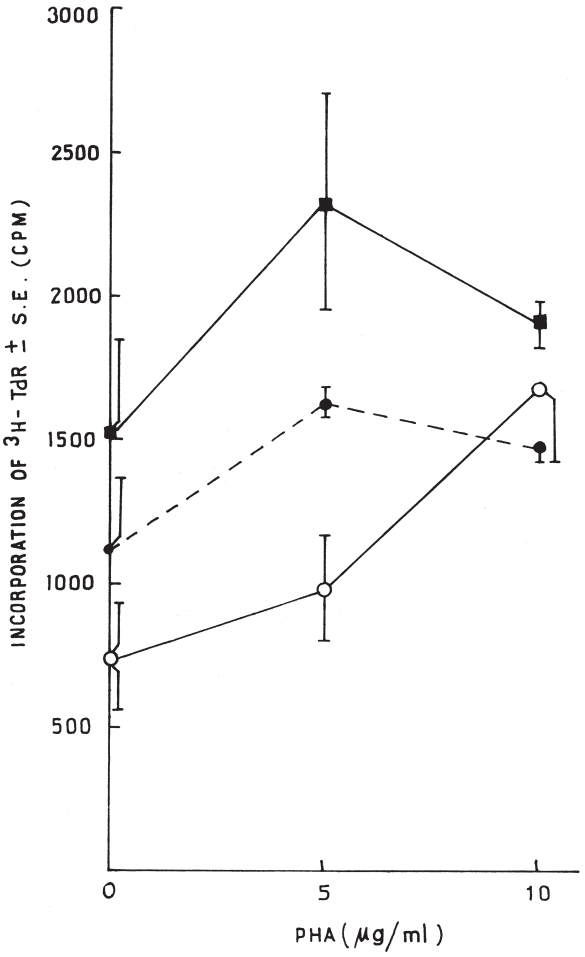

Fig. 1. Incorporation of ^{3}H-TdR (radioactive thymidine) by T lymphocytes of normal (○------○), alcoholic (●----●) and zinc supplemented alcoholic (■----------■) subjects after stimulation *in vitro* with different doses of PHA. CPM, (count per minute).

circulating and tissue alcohol level. Besides, the reported radio-protective effect of zinc and thiols (34) implies that organic zinc salts may, alone or in conjunction with thiols reduce a wider spectrum of tissue injury caused by free radical mediated mechanisms. We have discussed earlier about free radical mediated liver injury in the alcoholics (7). The reduction in the level of blood dehydroascorbic acid also stresses the same point. Carbon tetrachloride treated rats showed hepatic cell nuclear DNA fragmentation which could be reversed by the administration of zinc (35). Thus it is probable that both blastogenesis and the increased rate of DNA synthesis in alcoholics after zinc therapy could be a direct effect of zinc. The action of zinc also could be mediated by ascorbate/dehydroascorbate system through its antioxidant property.

A tendency of slight reduction in the level of serum immunoglobulin is reflected in the reduction in the serum level of both β and γ globulin. Although the T:B cell ratio remains almost unaltered, the percentage of rosette forming cells increase significantly after the supplementation. This is possibly due to some kind of change in the surface condition of the T cells.

The significant increase both in blastogenesis and the rate of DNA synthesis (Fig. 1) after the supplementation is suggestive of a possible change in the state of activity of the T cells of the alcoholics towards normalcy. The pattern of DNA synthesis of the T lymphocytes (Fig. 1) after the therapy however indicate that the cells are possibly in such a state that incorporates ^{3}H-TdR better than normal cells. That is why the c.p.m (count per minute) values are higher even without PHA stimulation.

It is quite evident from the observations that zinc therapy has a stimulating effect on the cell-mediated immune reactions of the alcoholics. Since zinc is necessary for the function of a wide variety of different enzymes involved in cell metabolism and cell division, there may be many possible mechanisms. Recent studies in experimental animals indicate that nucleoside phosphorylase which is essential for T Iymphocyte function may be zinc dependent (36). Thus it is possible that the stimulating effect of zinc on T cell mediated immune function may be affected through the activity of nucleoside phosphorylase.

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