Effect of Zinc Deficiency on Chromosomal Abnormalities in Mice

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ÖZKUL, Y., DURSUN, N., ERENMEMİŞOĞLU, A., SÜER, C. and SAATÇI, Ç. *Effect of Zinc Deficiency on Chromosomal Abnormalities in Mice.* Tohoku J. Exp. Med., 1996, 179 (4), 247-251 — In the present study we investigated the effect of zinc-deficiency on chromosomal abnormalities and mitotic index, and the interaction of zinc-deficiency and methotrexate. Therefore, two zinc-deficient and two zinc-adequate diet received mouse groups were constituted. Methotrexate was injected to one of zinc-deficient and one of zinc-adequate diet groups. There were no structural and numerical abnormalities in all groups. However, mitotic index was significantly lower than other groups in zinc-deficient and methotrexate administered mice. ——— zinc deficiency; chromosome; mitotic index

Zinc is a common element in the human environment and constitutes an important trace element intervening in many biological processes (Leonard et al. 1986). Zinc plays an important role in the biosynthesis of nucleic acids, mainly by affecting thymidine kinase and DNA polymerases. Toxicity of zinc is low, zinc deficiency represents, however, a hazard for human health. It is known that, zinc deficiency causes a reduction in the specific activity of many zinc-requiring enzymes (Kirchgessner et al. 1976). The most important role is attributed to thymidine kinase (Prasad and Oberleas 1979) which phosphorylates thymidine prior to its incorporation into DNA during replication, and the lack of which can reduce the rate of DNA replication and cell proliferation (Sanstead and Rinaldi 1969). Additionally, it is known that methotrexate (MTX) is an analogue of folic acid with higher affinity for dihydrofolate reductase than folic acid, so that the synthesis of folic acid is potently inhibited. Folic acid is required for the production of thymidine which in turn is required for DNA synthesis. In this condition, it would be expected on interaction between zinc deficiency and MTX administration. Studies on mutagenicity of zinc suggest strongly that zinc does not represent a mutagenic risk (Miyagi et al. 1977) and does not induce misrepair-

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ing in vitro between nucleic acid bases (Murray and Flessel 1976). Whereas, no information appears to be available on chromosomal abnormalities of zinc deficiency (Leonard et al. 1986) and MTX administration in zinc deficiency. The purpose of this study has aimed to explain these questions partially.

MATERIALS AND METHODS

Swiss albino mice ($n=20$) of both sexes, 90 days old, weighing from 22–26 g and bred in our laboratory were subjected to the present study. Animals were maintained according to "Guide to the care and use of experimental animals" by the Canadian Council on Animal Care (CCAC 1984). All mice were divided equally into four experimental groups of five mice each. Two groups of mice were fed with a zinc-deficient diet. The others were fed with a zinc-adequate diet. Diets were prepared as previously described (Bieri et al. 1977; Dursun and Aydoğan 1994). The zinc-deficient diet contained 2.8 mg/kg zinc and the zinc adequate group was fed 51.6 mg/kg zinc as calculated. While the zinc deficient group was allowed free access to deionized-distilled water, the zinc adequate group was allowed free access to the tap water. Food and water were available continuously ad libitum. After eight weeks of the dietary treatment, 0.5 mg/kg MTX was injected to one of zinc-deficient and one of zinc-adequate diet groups intraperitoneally as a single dose (Schrieber et al. 1985). MTX injection was not applied to the other groups. Twenty four hr after the MTX injection, 4 mg/g colchicine was injected to all mice intraperitoneally to block the cell division in metaphase. Two hr after the colchicine injection, mice were killed by decapitation and blood was collected and then, plasma was separated by centrifugation. The plasma levels of zinc were determined by atomic absorption spectrophotometry (Z-8000 Model, Hitachi, Tokyo) as described by Bonilla (1978), with minor modification using an air-acetylene flame after a 1:3 dilution of plasma with deionized-distilled H₂O. Standard solutions were prepared with deionized water from a certified stock of zinc solution containing 1 g zinc/liter. Standard solutions produced identical standard curves. The femur was dislocated from each mouse and bone marrow preparations were performed by the method as described by Schmid (1976), with some modifications. Bone marrow cells were incubated in 0.07 M KCl for 20 min at 37°C and centrifuged at 200 g for 5 min. Then the pellets were resuspended in 6 ml of 3:1 methanol: acetic acid fixative 3 times. They were prepared and stained with 5% Giemsa in Gurr's buffer for 6 min at room temperature. Fifty metaphase cells were examined for each mouse. Numerical and structural abnormalities were evaluated. Hypodiploid cells were not evaluated because of some known reasons (chromosome loss or preparational artifact). Proportion of aneuploid cells were calculated as twice the proportion of hyperdiploid cells. Additionally, one thousand cells were counted for each mouse to determine the mitotic index and metaphase cells were included only.

Data were analyzed using the z-approximation to compare independent

proportions (Dawson-Saunders and Trapp 1990). The level of significance was set at 0.05.

RESULTS

During the 8-weeks period of the experiment, evident external symptoms of zinc deficiency, including loss of appetite, alopecia skin lesions, and decreased statural growth were observed. The other difference between the zinc deficient and zinc adequate groups, the decline in diet consumption in zinc deficiency groups was observed (Table 1). There were no structural and numerical abnor-

TABLE 1. *Effects of zinc deficiency on body weight and food intake*

Groups	Food intake g/day/group	Body weight (g ; mean $\pm$ S.D.)	
		Before experiment	At the end of experiment
Zinc-adequate	12	25.2 $\pm$ 0.95	20.8 $\pm$ 0.97
Zinc-adequate-MTX	12	22.7 $\pm$ 0.89	19.9 $\pm$ 1.22
Zinc-deficient	7	24.3 $\pm$ 1.30	18.4 $\pm$ 1.41
Zinc-deficient-MTX	8	23.8 $\pm$ 1.29	17.2 $\pm$ 1.26

MTX, Methotrexate.

TABLE 2. *Distribution of diploid and hyperdiploid metaphase chromosomes in mouse bone marrow*

Groups	Total metaphase cells	Structural abnormalities				Numerical abnormalities		
		Break	Dele- tion	Gap	Ring	Hypo- diploid ≤ 39	Normal 40	Hyper- diploid ≥ 41
Zinc-adequate	264	1	—	2	—	5	257	3
Zinc-adequate-MTX	259	—	—	3	—	9	254	2
Zinc-deficient	280	2	1	4	—	11	247	5
Zinc-deficient-MTX	292	1	—	4	—	12	252	4

MTX, Methotrexate.

TABLE 3. *Mitotic index proportions*

Group	Proportion %
Zinc-adequate	3.90
Zinc-adequate-MTX	2.92
Zinc-deficient	2.86
Zinc-deficient-MTX	1.56*

Significant difference between Zinc-adequate and Zinc-deficient-MTX ($p < 0.05$).

MTX, Methotrexate.

TABLE 4. *Plasma zinc levels of mice*

Groups	Plasma zinc level ($\mu\text{g/ml}$) (mean $\pm$ s.e.)
Zinc-deficient	1.14 $\pm$ 0.23*
Control	2.42 $\pm$ 0.51

* $p < 0.05$

malities in the two zinc deficient groups (one of these received no MTX) and the zinc-adequate-MTX groups when they were compared to the zinc-adequate group (control) (Table 2, $p > 0.05$).

The mitotic index was significantly lower in zinc deficient-MTX group than the others ($p < 0.05$). The same parameter was also lower in the zinc-deficient without MTX group and the zinc-adequate-MTX group, but these differences were not statistically significant (Table 3, $p > 0.05$).

Plasma zinc levels of mice are given in Table 4. Plasma zinc levels of zinc-deficient mice were significantly lower than control ($p < 0.05$).

DISCUSSION

We could not get any information on chromosomal abnormalities of zinc deficiency in the literature. Our results suggest that zinc deficiency did not cause structural and numerical abnormalities in chromosomes of mice and it has no significant effect on the mitotic index. However, zinc-deficient and MTX treated animals had lower mitotic indices than the other groups ($p < 0.05$). Besides, Back et al. (1993) reported that the permeable zinc chelator blocked DNA synthesis in NIH 3T3 fibroblasts and upon addition of 11 microM zinc DNA synthesis was restored even after zinc chelator. In view of these findings, it was suggested that zinc deficiency may cause a sensitization to the effect of cytotoxic agents (perhaps mutagenic agents, too). This condition is expected because the role of zinc on DNA replication and cell proliferation via thymidine kinase has been known (Prasad and Oberleas 1976). Zinc is essential for the activity of superoxide dismutase activity, therefore the zinc deficiency in mice may cause to increase of free radical which may be an important problem (Coudray et al. 1991). On the other hand, MTX is a folic acid analogue and impairs the production of thymidine under these conditions, a decrease of the mitotic index may be expected. But in this study, only one dose of MTX has been assayed. In the preliminary studies doses higher or lower than 0.5 mg/kg MTX were also examined. But desired results could not be obtained. While, doses lower than 0.5 mg/kg of MTX had not any significant effect on mitotic index, doses higher than 0.5 mg/kg MTX have suppressed mitotic index strongly. In this condition the effect of zinc deficiency on mitotic index might be covered when it was administered at a dose higher than 0.5 mg/kg MTX. Although, according to our results, MTX alone appeared not to

have a forceful effect on mitotic index, in our opinion this condition may be related to MTX dose as discussed above. However, decreasing of mitotic index is clear in the zinc-deficient and MTX administered mice and it is not surprising.

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