TISSUE MALONDIALDEHYDE LEVELS IN ZINC DEFICIENT RATS

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SUMMARY: Free radicals have recently acquired great importance in pathogenesis of an array of diseases. In this report we present the results of two series of rat experiments in which tissue concentrations of malondialdehyde and zinc were investigated. In the first series of experiments the animals were given normal rat chow and tap water ad lib while the second group received zinc deficient diet and deionized water. Signs of zinc deficiency developed beginning the end of two weeks at which time the animals were sacrificed and the samples taken. Tissue zinc and malondialdehyde (MDA) contents were determined.

It was found that the zinc content in the kidney and the liver was significantly reduced in the experimental group ($p<0.05$). MDA concentrations were however higher in the brain and the heart of the experimental animals ($p<0.05$).

Key Words : Zinc deficiency, free radicals, malondialdehyde, lipid peroxidation.

INTRODUCTION

Free radicals have been the focus of attention during the last two decades. As a result voluminous literature has accumulated (1-3). A portion of them supplied new information concerning the role of free radicals in pathogenesis of a large array of diseases ranging from pathological conditions secondary to ischemia (4-6) to bronchopulmonary dysplasia (7,8) from pancreatitis (9) to uveitis (10) and from blood-brain barrier alterations (11) to tumor promotion (12-14), from traumatic paraplegia (15) to cerebral vascular injury (16), and from liver diseases (17,18) to rheumatoid arthritis (19), and to chronic renal failure (20).

Review of literature however indicates that concerning the role of free radicals in zinc deficiency diseases there presently are several research groups actively producing valuable new knowledge which simultaneously has increased many questions to be answered. We therefore planned the experiments reported in this communication where the results of such a study are presented.

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MATERIALS AND METHODS

This study was performed on 12 rats 2-3 weeks old, of either sex. Their average weight was 67 gr. They were separated into two groups. The first, being the control series (n=6) which were maintained on normal rat chow ad libitum and had free access to tap water. The experimental animals formed the second group (n=6).They were housed in stainless steel cages and were given zinc deficient diet containing only very low amounts of zinc (18) and de-ionized water in glass containers. This diet is sufficient to produce the first symptoms of zinc deficiency within the second week of its application.

Upon development of the full picture of zinc deficiency, blood samples were removed using disposable syringes and glassware washed with de-ionized water to prevent trace element contamination.

Table 1: Malone dialdehyde (MDA) and zinc concentrations of tissues obtained from experimental (Exp) and control (Cont) series of animals.

	Liver	Kidney	Lung	Brain	Heart	Spleen	Femur bone	Femur muscle	Femur skin	Skull Bone	Eye
M.D.A. (Exp) n.mol/gr	144 ± 4.7	154 ± 12.7	1349.8	274 ± 9.0*	162 ± 9.4	-	-	-	-	-	-
M.D.A. (Cont) n.mol/gr	154 ± 6.0	1596.4	1513.8	224* ± 4.2	138* ± 3.6	-	-	-	-	-	-
Zn (Exp) μg/gr	90* ± 8.5	66*3.4	-	79 ± 7.4	-	88 ± 19.7	146 ± 11.0	48 ± 9.5	43 ± 6.9	129 ± 9.0	48 ± 4.7
Zn (Cont) μg/gr	111* ± 12.4	146* ± 14.4	-	75 ± 11.3	- ± 3.9	97 ± 20.6	175 ± 8.9	65 ± 13.6	71 ± 11.3	184 ± 11.3	42 ± 1.2

*Indicate statistical significance between related groups, P<0.05.

Tissue aliquots were taken after exsanguination, from the brain, the scull, the bone, the lung, the kidney, the heart of the animals. The excess blood was blotted off and the tissue samples were weighted using a Nettler scale. They were then dissolved in concentrated nitric acid of known volume. Zinc content of these samples and of the serum were then determined, utilizing atomic absorption spectrophotometer (Perkin Elmer Model 103).

Serum malondialdehyde was determined using the method of Uchiama an Mihara (21). Tissue samples, taken for malondialdehyde determination, were homogenized and subjected to procedures as outlined before (21).

RESULTS

Clinical Observations

Clinical signs and symptoms of zinc deficiency developed in the experimental rats starting the second week and gradually became aggravated. The animals finally lost their alertness and became disinterested towards the surroundings. Their usual acute responses to minor stimuli disappeared and latter, remaining totally motionless at one corner of the cage, they became entirely apathetic. Starting from the vertex calvarium and upper most areas of shoulders alopecia appeared and gradually involved large areas. Loss of appetite and diarrhea supervened later.

Serum zinc levels

The blood samples removed on the 21st day of the experiment uncovered that the mean serum zinc level was 88.0±2.9 μg/dL compared to that of the control group, 113.0±5.6 μg/dL. The difference between the means of these two series of figures was statistically significant ($p<0.05$).

Tissue malondialdehyde contents

Mean values and standard errors of malondialdehyde content of the aliquots taken from the organs are given in Table 1 in nM/g of tissue. As can be seen, the malondialdehyde content of the brain and the heart were significantly above those of control animals ($p<0.05$). Malondialdehyde contents of the other organs of the experimental series however were not significantly different compared to those of the control animals ($p>0.05$, Table 1).

DISCUSSION

Diagnosis of zinc deficiency in these animals was confirmed by the clinical and laboratory findings described above.

The zinc and malondialdehyde (MDA) contents of several organs are presented in Table 1. Tissue zinc contents appear significantly different in the liver and the kidney ($p<0.05$) while MDA contents were different in the brain and the myocardium ($p<0.005$). Review of the remaining figures suggest the possibility of these differences in some other organs may reach to statistically significant levels in a more homogenous or larger

series. Such experiments are presently being performed in our laboratories.

It is rather early to place much importance on these findings. They should not be overlooked however, especially because of that the fact free radicals are gaining importance in the pathogenesis of so many heretofore unexplained diseases. Among these Chvapil and his group are the early contributors to this topic. They concluded that enhancement of lipid peroxidation by several noxious agents of physical, chemical and biological nature is a common denominator of various kinds of tissue injury. The same authors have presented evidence that zinc inhibits the endogenous as well as exogenous lipid peroxidation both in vivo and in vitro conditions (22-23).

Richard *et. al.* found that in patients undergoing hemodialysis that plasma zinc level was reduced while simultaneously malondialdehyde was increased (20). Hu and Chen observed in rats with induced liver damage secondary to D-galactosamine that MDA and oxidized glutathion were both elevated but reduced glutathion and zinc levels were lowered (23).

Hu and Chen investigated the free radicals, trace elements and neurophysiological function in rats with liver damage induced by D-galactosamine after which relative free radical concentration, MDA and oxidized glutathion and iron levels were elevated but reduced glutathion was decreased. Concurrently zinc, copper, manganese and selenium contents in liver were significantly reduced (24).

Huang studied lipid peroxides in patients with liver carcinoma. They noted that necrotic hepatic cell content of lipid peroxides were high and Zn, Cu and manganese contents were low (25).

Coudray showed that production of oxygen free radicals can be stimulated by excess iron, cadmium and nickel. Inversely copper, zinc and selenium inhibit production of the radicals either via their own action or by means of antiradical metalloenzymes. They found that experimental zinc deficiency caused a slight decrease of super oxide dismutase activity accompanied by increased production of peroxidated lipids. It is also important to add that ethanol administration caused a significant elevation in the levels of peroxidated lipids in the heart (26).

Cao and Chen performed zinc deficiency experiments in mice and observed that lipid peroxidation increased simultaneously however Cu-Zn SOD activity were reduced. It was further noted that zinc supplementation caused return of these abnormalities towards normal (27).

Gupta *et. al.* studied the lipid peroxides in the nervous system of rats fed a zinc deficient diet. Authors found the peroxides increased in several regions of the brain and spinal cord while superoxide dismutase was reduced in cerebrum, cerebellum, hypothalamus, hypocampus, brainstem and spinal cord (28).

Our results support the observations summarized above (20-28) suggesting that zinc deficiency is followed by excessive production of free radicals as expressed by a significant rise in MDA levels in tissues.

Another interesting aspect of our findings is that both MDA and zinc contents of various tissues obtained from our animals revealed quite a wide variation from one another. This observation deserves further consideration. In fact the studies of Dilley *et. al.* (29) may bring some explanation to this point. These authors observed that lung microsomes are much more resistant to peroxidation in vitro than those of liver and kidneys. The ratio of vitamin E to peroxidizable lipids was six times higher in the lungs than in the liver and kidneys. This relatively high amount of vitamin E might better protect lung membrane lipids from peroxidation. Jackson *et. al.* on the other hand observed that the skeletal muscle is much more resistant to peroxidation and ischemia compared to the heart, liver and intestines (30). Under the light of these studies we may consider that the zinc existing within the cells may function much in the same way as the vitamin E and other antioxidants. The fact that the normal rats' tissues contained less MDA in experiments animals may constitute an important evidence for this contention. This conclusion however needs further confirmation.

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