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Zinc Nutritional Status and Response to Lethal Level of Ozone Exposure in Rats

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A number of attempts have been made to discover the chemicals which may provide protection against the lethal effects of the air pollutant ozone. FAIRCHILD et al. (1959) reported the protective effect of sulfhydryl containing compounds against ozone and nitrogen dioxide in mice. PAGNOTTO and EPSTEIN (1969) demonstrated that various quinones provided protection against ozone toxicity in mice. GOLDSTEIN et al. (1972) showed similar results, i.e., decreased mortality upon ozone exposure, by pretreatment of rats with p-aminobenzoic acid. Protective effects of these chemicals on ozone toxicity was believed to be mediated through the antioxidant character of the chemicals. Ozone toxicity is known to proceed partly through lipid peroxidation.

CHVAPIL et al. (1972, 1973, 1974) reported the stabilizing effect of zinc on biomembranes in general. They showed, by *in vivo* and *in vitro* studies, that lipid peroxidation in rat liver stimulated by CCl_4 was substantially decreased by zinc pretreatment. They also showed that pretreatment of mice with a low dose of zinc (3 uM/kg body weight by gavage for 3 days prior to NO_2 exposure) prevented the development of lung edema induced by NO_2 exposure.

These findings raise the possibility that the nutritional status of zinc in animals may affect the response of animals to lethal levels of ozone exposure. With this concept in mind a study was carried out to explore the possibility of using zinc supplementation as a means of protection of animals from ozone toxicity.

METHODS

Animal and Diet Regimen

Weanling male Sprague-Dawley rats, 21 days old, weighing 50-60g were maintained individually in stainless steel cages and given free access to distilled-deionized water.

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In the first experiment the rats were randomly divided into three groups. Group A (zinc-deficient) received ad lib. commercially available zinc-deficient diet which contained 1.3 ppm zinc by analysis. The diet was purchased from Bio-Serv, Inc., Frenchtown, New Jersey 08825. The diet contained the following (g/kg diet): dextrose, 631; cellulose, 30; egg white solids, 200; corn oil, 100; salt mix, 37; Vitamin mix. No phytate was present in the diet. Group B (isocaloric control) received the zinc-deficient diet supplemented with zinc carbonate to raise the zinc concentration in the diet to 55 ppm. Rats in this group were pair-fed the 55 ppm zinc diet in an amount equal to that consumed by their pair-mates in Group A. To check the nutritional adequacy of the diet another group of animals (Group C) was provided with the same diet as Group B ad lib. At the end of three weeks feeding, all the rats were exposed to ozone simultaneously.

In the second experiment, a fourth group of animals (Group D) was added to the study and the feeding period was increased to four weeks. The diet regimens of Groups A, B, and C were identical to those of the first experiment. Group D (isocaloric control) received the zinc-deficient diet supplemented with zinc carbonate to raise the zinc concentration in the diet to 550 ppm. Rats in Group B and D were pair-fed according to the diet intake of their pair-mates in Group A. At the end of four weeks about one half of the animals from Groups A, B, and D were randomly selected and exposed to ozone. The rest of the animals were used for tissue zinc analysis. All the rats in Group C were exposed to ozone.

Ozone Generation and Exposure Conditions

Food was withheld from the night before the ozone exposure but water was available at all times. In each experiment rats were exposed simultaneously to 10 ppm ozone in the stainless steel chamber. Ozone was produced in oxygen in an electric arc discharge ozone generator and diluted with filtered room air. Ozone concentrations in the chamber were monitored in 1 hour intervals by the neutral buffered potassium iodide technique (SALTZMAN and GILBERT 1959). Exposure times required for death of rats were recorded in minutes.

Tissue Zinc Analysis

Rats were killed by decapitation and blood samples were collected from the head wound. Lung and liver were excised, trimmed of connective tissue, dried by blotting with filter paper, and weighed. The tissues were then placed in polyethylene bags and kept in a freezer until the analysis.

10 ml of equal parts concentrated nitric acid and perchloric acid were used for wet ashing of samples. Detailed procedure of wet ashing was described previously (KANG et al. 1977). Zinc concentrations of the digest were determined by a Perkin Elmer 303 atomic absorption spectrometer equipped with a three-slot burner

head, a hollow cathode lamp, and air-acetylene flame. Commercially available zinc standard solution purchased from Fisher Scientific Co. (Fair Lawn, New Jersey 07410) was used to establish the calibration curve.

Statistical Analysis

The data were analyzed using Student's t Test for the comparison of means.

RESULTS

Rats fed zinc deficient diet ad lib. (Group A) for three weeks developed typical zinc deficiency symptoms such as loss of appetite, retardation of growth, and loss of body hair. Rats pair-fed with 55 ppm zinc diet (Group B) did not show any apparent abnormality except retarded growth due to restricted diet intake. Rats fed the 55 ppm zinc diet ad lib. (Group C) showed normal growth, which indicates the nutritional adequacy of the diet (Table 1). Zinc deficiency in Group A could be ascertained by comparison of mean daily diet intake of Group A and Group C (6.8g vs. 12.8g) and the mean body weights of Group A and Group C at the end of 3 weeks (82.3g vs. 179.9g). Upon exposure of the rats to 10 ppm ozone there was no significant difference in the survival time between Group A and Group B. Rats in Group A and Group B were maintained under identical conditions, including diet intake, excepting amounts of zinc in the diet.

The second experiment was conducted to confirm these negative findings. A fourth group (Group D) of rats was added to the study, and fed diets containing more zinc. In addition, all the rats were maintained for a longer period of time. Table 2 summarizes the results from the second experiment. Typical zinc deficiency symptoms were apparent among the rats fed the zinc-deficient diet (Group A). The body weight gains of Groups A, B, and C were similar to the corresponding groups in Experiment I. Rats on the 550 ppm zinc diet (Group D) showed no apparent abnormality except retarded growth due to restricted diet intake. Their body weight gain was similar to that of the rats fed 55 ppm zinc diet, which indicates that 550 ppm zinc diet was not toxic to the animals. Among the three groups A, B, and D there was no difference in survival time upon 10 ppm ozone exposure.

Tissue zinc concentrations of the rats fed varying amounts of zinc for four weeks, but not exposed to 10 ppm ozone, are shown in Table 3. Zinc content of blood and liver was significantly different among the three groups. However, no difference in zinc concentrations of lung tissue was observed among the three groups.

TABLE 1

Effects of Feeding the Zinc Diets for 3 Weeks on the Body Weight Gain and Survival Time of Rats Upon 10 ppm Ozone Exposure

	Diet Regimen	Body Weight[a] (g)	Mean Survival (min. ± SD)
A.	Zinc-Deficient (7)[b]	82.3 ± 2.8	422 ± 100
B.	Isocaloric Control, (7) 55 ppm Zn Diet	94.0 ± 6.0[c]	449 ± 161
C.	Ad libitum, (7) 55 ppm Zn Diet	179.9 ± 6.4[c,d]	283 ± 120[c,d]

a. Rats were weighed one hour prior to ozone exposure.
b. Numbers in parenthesis indicate number of rats used in each group.
c. Significantly different from Group A; $p<0.05$.
d. Significantly different from Group B; $p<0.05$.

TABLE 2

Effects of Feeding the Zinc Diets for 4 Weeks on the Body Weight Gain and Survival Time of Rats Upon 10 ppm Ozone Exposure

	Diet Regimen	Body Weight[a] (g)	Mean Survival (min. ± SD)
A.	Zinc-Deficient (7)[b]	77.7 ± 22	493 ± 257
B.	Isocaloric Control, (7) 55 ppm Zn Diet	93.6 ± 29	461 ± 124
C.	Ad-libitum, (4) 55 ppm Zn Diet	228 ± 33[c]	189 ± 142[d]
D.	Isocaloric Control, (7) 550 ppm Zn Diet	95.1 ± 30	361 ± 142

a. Rats were weighed one hour prior to ozone exposure.
b. Numbers in parenthesis indicate number of rats used in each group.
c. Significantly different from Group A, B, D; $p<0.05$.
d. Significantly different from Group B; $p<0.05$.

TABLE 3

Body Weight, Lung Weight, and Tissue Zinc
Content of Rats Fed Varying Amounts of Zinc for 4 Weeks

Diet Regimen	Body Weight[a] (g)	Lung Weight (g)	Zn Content (µg/g wet tissue)		
			Blood	Liver	Lung
A. Zinc-Deficient (8)[b]	76.8 ± 14.9	0.57 ± 0.03[c] 0.76 ± 0.13[d]	3.2 ± 0.5	18.5 ± 5.7	15.0 ± 1.5
B. Isocaloric Control (7)	96.0 ± 16.8	0.70 ± 0.20 0.75 ± 0.25	4.3 ± 0.8[e]	30.2 ± 6.1[e]	13.0 ± 3.2
D. Isocaloric Control (6)	97.0 ± 25.3	0.69 ± 0.12 0.76 ± 0.20	5.3 ± 0.6[e,f]	39.9 ± 7.1[e,f]	15.8 ± 2.3

a. Rats were weighed at the end of 4 weeks feeding.
b. Numbers in parenthesis indicate number of rats used in each group.
c. Mean organ weight ± standard deviation in grams.
d. Mean organ weight relative to body weight ± standard deviation in percentage.
e. Significantly different from Group A; $p<0.05$.
f. Significantly different from Group B; $p<0.05$.

DISCUSSION

The hypothesis that pretreatment of rats with dietary zinc could provide the stabilization of biomembranes and consequent suppression of the lethal effect of ozone was not supported in this study. Certainly the amount of zinc used for pretreatment could not explain the failure to observe the protective effect. In this study the rats were pretreated with zinc for longer periods of time (3 days in CHVAPIL et al.'s study vs. 3 or 4 weeks) and with three different levels of dietary zinc: 1.3 ppm; 55 ppm; 550 ppm. In CHVAPIL et al.'s (1974) study mice were pretreated with zinc by gavage for 3 days prior to NO_2 exposure.

Rats fed 55 ppm zinc diet ad lib. (Group C in Experiment I and II) had significantly shorter survival time upon 10 ppm ozone exposure than rats from other groups. However, direct comparison of this result with other groups would be invalid. The amount of diet intake of the rats in this group was not restricted, leading to body weight gain 2 to 3 times those of rats pair-fed zinc supplemented diets in an amount equal to that consumed by their pair-mates in the zinc deficient group. The purpose of including this group in each experiment was to ascertain the development of zinc deficiency in the rats fed the zinc-deficient diet by comparison of their mean daily diet intake and body weight gain to those of rats in this group.

Rats pair-fed zinc-supplemented diets gained more weight than rats fed the zinc-deficient diet (Group A) even though their amount of diet intake was restricted to be equal to that of their pair-mates in Group A. This effect of zinc deficiency on the efficiency of weight gain of rats was also reported by others (MACAPINLAC et al. 1966, OBERLEAS and PRASAD 1969, HSU and ANTHONY 1971).

The most plausible explanation for these negative findings of no protection of animals against ozone damage by zinc pretreatment might be the organ specificity for zinc. In this study zinc concentrations of blood and liver increased in proportion to the amount of zinc in the diet. However, in the lung tissue there was no significant change in zinc concentrations. It is evident that only certain tissues show a significant change in their zinc content as a result of deficiency or supplementation. PRASAD et al. (1967, 1971) also observed a decrease in zinc content only in bone, testes, esophagus, kidney, liver, and thymus of zinc-deficient rats. The lung being a primary target organ for ozone damage and non-sensitive to zinc pretreatment, one could not expect a protective role of zinc in the lung against ozone damage. The protection of rat liver from CCl_4 induced lipid peroxidation reported by CHVAPIL et al. (1973) by Zn pretreatment of animals could be due to increased zinc content in the liver as shown in this study. They also reported that in vitro lipid peroxidation in liver microsomes induced by CCl_4 was substantially decreased by zinc throughout a large range of concentrations (CHVAPIL et al.

1972).

Previous in vivo and in vitro animal studies by others have demonstrated a protective effect of zinc in the liver against lipid peroxidation. The present study, however, showed that zinc supplementation of animals cannot provide protection from ozone induced lung damage simply because zinc does not accumulate in the lung tissues, the primary target organ for ozone damage.

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