Inhibition of Tumor Growth by Dietary Zinc Deficiency

James T. McQuitty, Jr., William D. DeWys, Liberatore Monaco, William H. Strain, Charles G. Rob, Jean Apgar, and Walter J. Pories


SUMMARY

The effect of different levels of zinc intake on tumor growth was studied by implanting Walker 256 carcinosarcoma into weanling Sprague-Dawley rats maintained on laboratory chow or on a zinc-deficient synthetic diet. Three experimental groups receiving this synthetic diet were given 0, 50, or 500 parts per million, respectively, of zinc ion in the drinking water. The latter 2 groups were pair fed the amount eaten by the unsupplemented group. The survival of rats receiving the zinc-deficient intake was significantly increased compared with the other 2 groups on synthetic diets or the group on laboratory chow. Tumor growth was markedly decreased in the zinc-deficient group and slightly decreased in the zinc-supplemented groups compared with control rats on laboratory chow. The reduced tumor growth in the zinc-deficient group was highly significant in a test for specificity of tumor inhibition. Since dietary zinc deficiency inhibited tumor growth, this study demonstrates the importance of zinc for neoplasms. Further investigation is needed on the role of zinc, as well as other essential metals, for malignant proliferation.

INTRODUCTION

Zinc is essential for normal proliferative processes. The importance of zinc in wound healing has been reported from this laboratory (11, 12, 14) and confirmed by others (7, 13). The necessity of zinc for the normal development of the chick embryo (8) and the rat fetus (6) has also been emphasized.

In a study of the effects of trace elements on the antitumor activity of a bis(thiosemicarbazone), zinc was found to be necessary for tumor growth as well as carcass growth (10). However, that study did not include pair-fed controls, nor was an effect on survival reported. The present investigation was undertaken to evaluate further the effects of zinc ion in an experimental tumor system. Rats given injections of Walker 256 carcinosarcoma were maintained on a zinc-deficient synthetic diet and were provided different levels of zinc ion via the drinking water. Zinc deficiency was found to produce a specific inhibition of tumor growth and this was accompanied by a significant increase in survival, thus further demonstrating the importance of zinc for tumor growth.

MATERIALS AND METHODS

Sprague-Dawley male rats, purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass., were 21 days old and weighed 40 to 47 g at the start of the experiment. Rats were housed individually in stainless steel suspension cages and were fed laboratory chow or a zinc-deficient synthetic diet in glass feed cups with stainless steel tops. The experimental diet, which meets the nutritional requirements of the rat except for the zinc (9), is given in Table 1. Tap water or deionized distilled water was supplied ad libitum in clear glass bottles with Neoprene stoppers and stainless steel spouts (Table 2).

Walker 256 carcinosarcoma was used as an intramuscular implant. This tumor was received from A. D. Little, Inc., Boston, Mass., and is carried as a weekly intramuscular transplant in Sprague-Dawley rats. A tumor cell suspension was prepared by mincing tumor fragments through a cytosieve (U. S. Standard Sieve Series, 100 mesh/inch, Dual Manufacturing Company, Chicago, Ill.) and then suspending the cells in Hanks' balanced salt solution. Experimental rats were given injections of 10^7 cells in 0.2 ml in the right hindleg muscles.

The experimental animals were divided into 30 weight-matched sets of 4 and each member of the set was assigned to 1 of 4 experimental groups (Table 2). All rats were fed the assigned diet ad libitum for 4 days and then pair fed as indicated (Table 2). The tumor was injected on the 8th day after initiation of the experimental diet. The anteroposterior and mediolateral diameters of the tumors were measured with calipers daily until the death of the animal.
Table I

Composition of diet

<table>
<thead>
<tr>
<th>Dietary constituent</th>
<th>Composition (%)</th>
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<tbody>
<tr>
<td>Egg albumin</td>
<td>26.4</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>53.2</td>
</tr>
<tr>
<td>Corn oil</td>
<td>10.0</td>
</tr>
<tr>
<td>Mineral mix*</td>
<td>8.14</td>
</tr>
<tr>
<td>Vitamin mix*</td>
<td>1.0</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.5</td>
</tr>
<tr>
<td>L-Histidine hydrochloride</td>
<td>0.5</td>
</tr>
<tr>
<td>Santoquin antioxidant (dry)</td>
<td>0.02</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.22</td>
</tr>
</tbody>
</table>

* Mineral mix supplied the following minerals in mg/100 g diet: K2HPO4, 3383; CaCO3, 2161; NaCl, 1373; KCl, 446; MgCO3, 398; MgSO4, 253; FeSO4 · 7H2O, 102; MnSO4 · H2O, 16.7; CuSO4 · 5H2O, 5.5; NaF, 4; KI, 0.3.

One g mix supplied the following vitamins/100 g diet: inositol, 25 mg; niacin, 5 mg; calcium pantothenate, 2.0 mg; thiamine-HCl, 1.0 mg; riboflavin, 1.0 mg; pyridoxine-HCl, 0.45 mg; folic acid, 0.40 mg; biotin, 0.43 mg; vitamin A, 500 i.u.; vitamin D (Delstrol), 150 i.u.; vitamin B12, 2.0 µg; vitamin K (Klotogen F), 0.152 mg; vitamin E (Rovimix), 6.6 i.u.

Table II

Experimental design

<table>
<thead>
<tr>
<th>Group and designation</th>
<th>Dietary regimen</th>
<th>Water (ad libitum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chow</td>
<td>Laboratory chow, ad libitum</td>
<td>Tap</td>
</tr>
<tr>
<td>2. Zinc deficient</td>
<td>Experimental diet, ad libitum</td>
<td>Deionized distilled</td>
</tr>
<tr>
<td>3. Zinc adequate</td>
<td>Experimental diet, pair fed</td>
<td>0.05 g Zn++/l†</td>
</tr>
<tr>
<td>4. Zinc excess</td>
<td>Experimental diet, pair fed</td>
<td>0.5 g Zn++/l†</td>
</tr>
</tbody>
</table>

* Groups 3 and 4 were pair fed the amount consumed during the previous day by a paired member of Group 2, beginning on the 4th day of experiment.

† Zinc supplement stock solution was prepared by dissolving 16.79 g zinc acetate, Zn(C2H3O2) · 2H2O, in 1 liter of deionized distilled water containing 10 meq acetic acid. Stock solution was then diluted 1:100 or 1:10, respectively, in deionized distilled water for Group 3 or 4. The daily zinc intake would then be 1.0 or 10.0 mg, respectively, with an average water intake of 20 ml/day/rat.

The data of tumor size and host weight were tested for specificity of inhibition according to the method of Skipper et al. (15). For this test, the effect of caloric restriction per se on tumor growth was evaluated by comparing within each matched set the tumor size and host weight of the zinc-adequate pair-fed rat (caloric restricted) with the tumor size and host weight of the chow-fed rat. The ratio of the cube of tumor diameter of these 2 was plotted as a logarithmic function of the host weight difference (Chart 4). A regression line determined from these data (see Ref. 15 for method of determining regression line) then describes the effect of caloric restriction per se on tumor size. The effect of the zinc-deficient diet was similarly evaluated by comparing within each set the tumor size and host weight of the zinc-deficient rat with the chow-fed rat. The criteria of Skipper et al. (15) were then applied to the data. The use of the cube of tumor diameter instead of tumor weight as used by Skipper et al. (15) is supported by the known proportionality between the cube of tumor diameter and tumor weight. Differences in survival were tested for significance with the Mann-Whitney U test.

Chart 1. Survival response with different levels of dietary zinc. Median survival was 11.0 days in laboratory chow, zinc-adequate, and zinc-excess groups and 19.0 days in the zinc-deficient group (p < 0.001).

Chart 2. Tumor growth rate (mean ± S.E.). Tumor growth was markedly inhibited in the zinc-deficient group and slightly inhibited in the zinc-adequate and zinc-excess pair-fed groups.
RESULTS

The survival response is shown in Chart 1. Survival of the chow, zinc-adequate, and zinc-excess groups was quite similar; in all groups median survival was 11.0 days. There was a suggestion of earlier deaths at the lower end of the survival curve for the animals being pair fed (and thus caloric restricted) compared with the chow group. The survival of the zinc-deficient group was prolonged (median, 19.0 days) compared with the other groups ($Z = 5.24; p < 0.001$).

The tumor growth data are shown in Chart 2 and carcass growth data in Chart 3. The decreased tumor growth of the zinc-excess and zinc-adequate groups compared with the chow group was accompanied by a decreased carcass growth rate and reflects the decreased caloric intake of pair feeding. (In a separate experiment, no difference in tumor growth or carcass growth was observed between rats fed the zinc-adequate regimen ad libitum or laboratory chow ad libitum.) No difference was noted between the zinc-excess and zinc-adequate groups in either tumor growth or carcass growth, thus indicating that high levels of zinc were without measurable effect in this study.

A decreased tumor growth rate of the zinc-deficient group compared with the other 3 groups was noted (Chart 2). This was accompanied by a decreased carcass growth rate. However, when compared with the zinc-adequate group the tumor inhibition seemed out of proportion to the inhibition of carcass growth. As a further test of this, a specificity test (15) was applied to the data obtained on Day 8 after tumor implantation (Chart 4). The data of the zinc-adequate group were used as the caloric-restricted controls for this test. The data for the zinc-deficient group pass the criteria of specificity at the 99.7% confidence level (15) (Chart 4).

An interesting aspect of the zinc deficiency was the speed with which it developed. If anorexia is used as the criteria for zinc deficiency, these young rats were showing deficiency on Day 7 or 8 after initiation of the diet. This indicates that the zinc stores are rather small in the growing rat and also indicates that the onset of zinc deficiency coincided with the implanting of the tumor in this experiment.

DISCUSSION

Tumor growth may be inhibited by nonspecific factors such as caloric restriction or specific factors such as use of an active antitumor agent. The magnitude of tumor inhibition by caloric restriction in the model system used herein has been reported previously (4). The tumor inhibition noted in Groups 3 and 4 is of the order of magnitude expected with the degree of caloric restriction in-
volved in pair feeding. Also, caloric restriction would not be expected to lengthen survival (4), and no increase in survival was noted in Groups 3 and 4 compared with the chow-fed group.

In contrast, the tumor inhibition noted in the zinc-deficient group is thought to represent specific tumor inhibition. This is supported by the increase in survival and, also, by the mathematical evaluation for specificity. Previous studies have suggested that a survival time assay of drug effect may be more specific than a tumor weight inhibition assay (4). The increased survival noted with zinc deficiency compares favorably with the increased survival noted with several of the drugs currently in clinical use (4).

The mathematical test for specificity reported by Skipper et al. (15) is based on extensive experience with experimental evaluation of potential anticancer drugs (15). Our application of this test to the present data seems valid since the only known difference between the zinc-deficient and the pair-fed controls was the zinc intake. Although the synthetic diet may possibly contain some unidentified deficiency, a comparison of Groups 2 and 3 tests the effects of zinc deficiency. The degree of specificity noted for zinc deficiency also compares favorably with that observed with several of the drugs currently in clinical use (15). Thus, both the increase in survival and the mathematical test for specificity support the concept of significant specific tumor inhibition by zinc deficiency. These results then confirm and extend the previous studies of the requirement for zinc ion by tumor growth (10).

The requirement for zinc by growing tumor shown in the present study suggests a possible explanation for the observed decreased levels of zinc in sera or cells from human cancer patients (1–3, 5, 16, 17). Thus, serum zinc may be low because the element is selectively used by tumor at the expense of the host. Zinc levels may be low in cancer cells compared with normal because the available zinc is divided among the cells competing for it. Dennies et al. (3) have observed an inverse correlation between leukemia cell count and leukocyte zinc content. Whether zinc depletion could result in this way is not clear. Also, systematic studies of the effects of these decreased levels of zinc on tumor growth have not been reported. Davies et al. (2) state that no correlations could be made between decreased plasma zinc and the growth and spread of the neoplasm. The present results suggest that decreased levels of zinc might inhibit tumor growth.

These results of inhibition of tumor growth by dietary zinc deficiency have important implications. First, they suggest a unique approach for inhibition of neoplastic growth. Second, by pointing out the importance of one essential metal in tumor growth, they indicate the importance of investigating the role of other essential metallic elements in malignant proliferation (10).

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REFERENCES

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