Copper, Zinc, and Iron in Normal and Leukemic Lymphocytes from Children

Ugo Carpentieri, Jerry Myers, Larry Thorpe, Charles W. Daeussner III, and Mary Ellen Haggard

ABSTRACT

Copper, zinc, and iron were quantitated in the serum and lymphoid cells of the peripheral blood of healthy children and children with acute lymphocytic leukemia. Copper and iron concentrations in serum and cells were significantly higher and zinc concentration in the cells significantly lower in leukemic patients than in healthy donors, whereas the increase of zinc in the serum was not significant. The concentration of all minerals was higher in T-cell enriched preparations. There was a significant correlation between copper and iron and between copper and zinc, but not between iron and zinc in normal and leukemic lymphocytes. No correlation was demonstrated among the three minerals in the serum. There were no significant differences associated with ethnicity, age, sex, type of leukemia, or number of leukemic cells. However, a group of five children with non-B-, non-T-cell acute lymphocytic leukemia, nonreactive skin tests, and low serum immunoglobulins had high concentrations of copper and iron and low concentration of zinc in their leukemic cells. Since copper, zinc, and iron are associated with lymphocyte maturation and regulation of immune function, these new data will provide a tool for the study of the relationship between changes in concentrations of these metals and the modification of the immune response often present in hematologic cancers.

INTRODUCTION

Copper, zinc, and iron have been associated with normal lymphocyte maturation and regulation of immune function (1–8). Low levels of these minerals have been demonstrated in a variety of dysfunctions of the immune system; low serum concentrations of zinc, in fact, may be present in patients with hypogammaglobulinemia and defective cell-mediated immunity (1, 2); impairment of leukocyte function has been observed with copper deficiency (7) and lymphoid atrophy with impaired cell-mediated immunity has been identified in subjects with hypoferremia (8). Information on high concentrations of copper, zinc, and iron and their effect on lymphocyte function is also available (9, 10).

Serum concentrations of copper, zinc, and iron are modified in some cancers; serum copper concentrations may be increased in some leukemias (11–13) and lymphomas (14), whereas a decrease in iron and variations of zinc concentrations have been demonstrated in leukemia (12–13). Abnormal immune response has also been associated with these cancers; patients with Hodgkin’s disease are often anergic (15) and depression of humoral and cellular immunity has been seen in untreated leukemia (16).

These studies suggest a possible causal relationship between changes of copper, zinc, and iron concentrations and modifications of the immune response associated with hematologic cancers. A proper evaluation of this relationship, however, may be attempted only when data on serum concentrations of copper, zinc, and iron are combined with data on their concentrations in normal and malignant lymphoid cells. To the best of our knowledge, the only information presently available is limited to the zinc concentrations in normal lymphocytes in adult subjects (17) and patients with chronic lymphocytic leukemia (18).

In this study we report data on the quantitation of zinc, copper, and iron in lymphoid cells of healthy children and children with ALL. We also report observations on the potential relationship between changes in the cellular concentration of these minerals and lymphocyte function in certain patients with leukemia.

PATIENTS AND METHODS

Twelve children, aged 2–12 yr (mean age, 5.7 yr) admitted consecutively to our institution with a diagnosis of ALL were studied before any treatment was initiated. Five were black (two males and three females) and seven were caucasian (five males and two females). Twenty-one healthy children in the same age range (siblings when available), chosen at random (except for siblings) among those attending our well child clinic were also studied. Nine were black (five males and four females) and twelve were caucasian (eight males and four females).

The study was approved by the Institutional Review Board for Human Experimentation and informed consent was obtained in all cases.

History, physical examination, hemogram, and skin testing with Candida (1:250) and PPD were done on all subjects. Quantitation of serum immunoglobulins was also obtained from all children as were serum concentrations of copper, zinc, and iron. Diagnostic bone marrow aspiration was performed in children with leukemia.

Normal lymphocytes were isolated by Ficoll-Hypaque density gradient centrifugation (19) from morning specimens of peripheral venous blood of fasting healthy donors. Erythrocytes were removed by hypotonic shock (17), platelets by discontinuous sucrose gradients (19), and macrophages by incubation in Petri dishes with inactivated calf serum (20, 21). Cell viability was tested by the trypan blue exclusion method, contamination was tested by morphology, and macrophages by latex particle ingestion. T-cell enriched aliquots of lymphocytes were then obtained with mouse anti-human B-cell monoclonal antibody-coated polystyrene Petri dishes (20, 21) and percentages of B- and T-cells were calculated before and after enrichment with fluorescein-conjugated anti-human B- and T-cell antibodies. All antibodies were purchased from Cappel-Worthington Biochemicals, Malvern, PA. The cells were then diluted to 1 × 10⁷ cells/ml in phosphate buffered saline, pH 7.4, and digested overnight with an equal volume of concentrated nitric acid (Utrex; J. T. Baker Co.) at room temperature and for 4 additional h in a water bath at 60°C (17). Blanks (phosphate buffered saline and nitric acid in equal vol) were analyzed simultaneously. Standards were prepared as described, accounting for the matrix effect (17). The cell specimens and sera were stored at −20°C until assayed.

Quantitation of metals in serum and cells was performed with an AA spectrophotometer (ILLI-Model 551 with microcup assembly and full scale

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2 To whom requests for reprints should be addressed, at Department of Pediatrics, University of Texas Medical Branch, Rt. C-61, Galveston, TX 77550.
3 The abbreviations used are: ALL, acute lymphocytic leukemia; PPD, purified protein derivative of tuberculin.
TRACE MINERALS IN LEUKEMIA

expansion) (22). Plasticware was used during the entire procedure with careful elimination of external contamination (17). Serum metals were expressed in μg/ml and cellular metals as μg/10^10 cells. Intracellular metals were also expressed in ng/mg protein.

For leukemic patients the same protocol was used with the following modifications: identification of the type of leukemia (B-, T-, non-B, non-T-cell ALL) was obtained through the Pediatric Oncology Group, the percentage of B- and T-cells was not calculated, and T-cell-enrichment was not done.

Potential loss of intracellular minerals due to the isolation procedure was assessed in several randomly chosen healthy donors and patients with leukemia, by quantitation of copper, zinc, and iron in the initial specimen and in the several aliquots of cells and supernatant solutions obtained during the procedure. Supernatant solutions were concentrated in an oven at 135°C. Possible effects of the isolation procedure on T4/T8 ratio of lymphocyte subsets in healthy children were also determined with monoclonal antibodies (Ortho Diagnostic System, Raritan, NJ), according to the manufacturer’s indications.

Statistical evaluation included Student’s t test and correlation coefficient analysis.

RESULTS

Healthy Donors. Physical examination, hemogram, and serum immunoglobulin concentrations were normal for age (23). The skin test with Candida was positive after 48 h (>5 mm induration), whereas the PPD skin test was nonreactive.

Lymphocytes were 98% pure and 96% viable. T-cell percentages were 65–75% before and 90–95% after enrichment. B-cell percentages were 15–20% prior to and 3–4% after enrichment. T4/T8 ratio was not significantly altered after cell isolation (range, 2.1–2.6 before isolation versus 2.0–2.3 postisolation).

The values for serum and intracellular concentrations of zinc, copper, and iron in normal lymphocytes are summarized in Table 1. These values were 20–25% higher (P < 0.02) in T-cell-enriched preparations (Table 1, footnote e). There were no significant differences associated with ethnicity, age, sex, or number of cells.

There was no statistically significant correlation between serum metals and intracellular metals or between intracellular iron and intracellular zinc. However, there was a significant inverse correlation between intracellular copper and zinc (r = 0.6; P < 0.01) and a significant direct correlation between intracellular copper and iron (r = 0.8; P < 0.01).

Children with Leukemia. There were no significant differences in socioeconomic status, diet, and previous medical history between this group and the group of healthy donors. Physical examination, hemogram, and bone marrow aspirate were consistent with the diagnosis of ALL. There were two pre-B-, two T-, and eight non-B-, non-T-cell leukemias among this group of patients. Serum immunoglobulin levels were normal for age and Candida skin test was positive at 48 h in seven patients, whereas serum immunoglobulins were low normal (between one and two SDs below the mean for age) and Candida skin test was nonreactive in the remaining five patients (two caucasian males, two black females, and one caucasian female), all with non-B-, non-T-cell leukemia. PPD skin test was negative in all patients.

Lymphoid cells were 85–90% leukemic lymphoblasts and lymphocytes. The serum and intracellular concentrations of zinc, copper, and iron are presented in Table 1. There were no statistically significant differences in these concentrations associated with ethnicity, age, sex, type of leukemia, or number of leukemic cells. Ten–12% of these metals were lost during the procedure. This loss was significantly greater (P < 0.01) than that observed in the processing of normal lymphocytes (data not shown). All serum metals in patients with leukemia were higher than in healthy children, but the increase was statistically significant (P < 0.01) only for copper and iron. The increases in intracellular copper and iron were significant (P < 0.01), whereas the decrease in intracellular zinc was significant only when ex-

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Zinc (μg/dl)</th>
<th>Copper (μg/dl)</th>
<th>Iron (μg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy children (n = 21)</td>
<td>103 ± 22</td>
<td>114 ± 29</td>
<td>68 ± 26</td>
</tr>
<tr>
<td>Children with ALL (n = 12)</td>
<td>136 ± 48</td>
<td>328 ± 74</td>
<td>96 ± 24</td>
</tr>
<tr>
<td>P</td>
<td>not significant</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Table 1**

Zinc, copper, and iron in serum and lymphoid cells of healthy children and children with acute lymphocytic leukemia

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**Healthy children (n = 21)**

<table>
<thead>
<tr>
<th>Zinc (μg/10^10 cells)</th>
<th>Copper (μg/10^10 cells)</th>
<th>Iron (μg/10^10 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>72 ± 6 (57-96)</td>
<td>15 ± 4 (4-40)</td>
<td>2.5 ± 0.2 (0.7-4)</td>
</tr>
<tr>
<td>Healthy children (n = 21)</td>
<td>(380-610)</td>
<td>(26-260)</td>
</tr>
<tr>
<td>Children with ALL (n = 12)</td>
<td>54 ± 9 (10-90)</td>
<td>52 ± 16 (29-71)</td>
</tr>
<tr>
<td>P</td>
<td>not significant</td>
<td>&lt;0.01</td>
</tr>
</tbody>
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**Note:**

- μg/10^10 cells: micrograms per 10^10 cells.
- ng/mg protein: nanograms per milligram protein.
- P: Probability.

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pressed in ng/mg protein (Table 1).

A statistically significant direct correlation was found between serum copper and intracellular copper \((r = 0.7; P < 0.01)\), between serum iron and intracellular iron \((r = 0.7; P < 0.01)\), and between copper and iron in the cells \((r = 0.8; P < 0.001)\). A statistically significant inverse correlation was found between intracellular zinc and intracellular copper \((r = 0.6; P < 0.01)\). No other significant correlation was demonstrated.

The five patients with non-B-, non-T-cell leukemia (Table 2), low serum immunoglobulins, and negative skin test did not differ significantly from the other seven in age range, diet, socioeconomic status, and medical history, but revealed high intracellular concentrations of copper and iron and low concentrations of intracellular zinc. Serum concentrations were in the high ranges for all three metals.

### DISCUSSION

Serum concentrations of zinc, copper, and iron in healthy children reported in this study are similar to previously published values (23). In addition the variations of serum copper and iron in patients with acute lymphocytic leukemia have been described before (11–13). However, the changes in serum zinc concentration found in some leukeemic patients (12–13) were not observed in ours. This discrepancy is possibly due to differences in diet and time of blood sampling since zinc is subject to a circadian rhythm and its concentration is sensitive to dietary intake (24). No mention of these factors is made in the other study (13).

The values for lymphocyte content of zinc in our healthy children were lower than those reported previously (17, 18). Since our donors were healthy and on a balanced diet, the discrepancy may be due to the differences in age (pediatric donors in our study versus adult subjects in the others). It may also reflect, however, the careful and complete removal of macrophages and platelets (which contain discrete amounts of zinc) (22) from our samples before the assay; when in fact platelets were removed results similar to ours were obtained (22).

We were unable to compare the values for iron concentration in the lymphocytes of our healthy children with those reported by others (25) since those investigators studied normal lymphocytes in culture and made no mention of the iron content of the media and possible loss of metal from the cells. To the best of our knowledge, no data are available for freshly isolated lymphocytes. Similarly, no comparison can be made between our results for copper and zinc content of normal lymphocytes and those reported previously (26, 27) since the latter results were determined on mixed populations of WBC without separation of lymphocytes from neutrophils.

The lack of correlation among the serum concentrations of copper, zinc, and iron and between the serum level of each of these metals and their content in normal lymphocytes demonstrated in this study suggests that their blood concentrations may not be interdependent at variance with other reports (28, 29), and that an active regulation of their intracellular level, possibly at the membrane level, may exist (29–31). In this context, the higher mineral content of T-cell-enriched preparation may also be due to membrane regulation, perhaps related to the greater diversity and number of functions of T-cells as compared to other types of lymphocytes (32).

The changes in the mineral concentrations of ALL cells identified in our patients have not been described before; only decrease of intracellular zinc in chronic lymphocytic leukemia has been reported (18). We speculate that the changes in ALL may represent either a characteristic of an abnormal clone population of lymphocytes or a phase of the maturation process of normal lymphoid elements. The high turnover of these cells (33) may explain the increased concentrations of minerals in the serum, although anemia and poor general nutrition, conditions frequent in acute lymphocytic leukemia, may be additional factors (34). Changes in metallothioneins should also be considered (29).

The causes for a greater loss of metals from leukemic cells than from normal lymphocytes during the isolation process are not readily apparent. Modification of the cell membrane may be one factor which, in turn, may explain the direct correlation between copper and iron concentrations in the sera and lymphoid elements of children with ALL which was not present in healthy donors.

Few children with ALL and low serum immunoglobulins (35) and, in one case, with potent suppressor activity of neoplastic T-cells (36), have been identified in the past, but a study on the mineral content of their cells was not done. The identification of some leukemic children among our patients with the simultaneous presence of low concentration of serum immunoglobulins, absent cutaneous reactivity, and rather extreme variations of intracellular metals (low zinc, high copper and iron) is provocative. In the absence of other identifiable causes it is tempting to conclude that a relationship may indeed exist between fluctuations of mineral concentrations and cell function and/or maturation in some patients. Since this conclusion may have therapeutic implications (37, 38) more stringent criteria of evaluation and a study on a larger number of patients are recommended.

### ACKNOWLEDGMENTS

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### REFERENCES


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