Changes in Urine Polyamines in Childhood Leukemias*

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ABSTRACT

Urine polyamine: creatinine ratios (nm per mg) measured in eight children with active hematologic cancers were compared with those of age-matched controls and children with hematologic cancer in remission. Polyamine: creatinine ratios in the children with active disease were significantly higher than those of the controls (p < 0.0025) and of the children in remission (p < 0.0025). Putrescine: creatinine ratios were, in general, higher in children with hematologic tumors than in those with solid tumors. Urinary polyamines are thought to reflect variations in bone marrow polyamine content and have been postulated to be an indicator of clinical status.

Introduction

Rapidly growing or proliferating tissues, including human malignant tumors, are known to contain high concentrations of polyamines.6,16,17 As early as 1853, leukemic spleen was shown to be rich in spermine.8 However, the first successful application of polyamine determinations as indicators of malignancy was the demonstration by Russell et al of increased serum and urine polyamines in cancer patients.25,26 Rennert et al reported a correlation between bone marrow polyamine concentration and percentage of malignant cells present in the bone marrow in children with leukemia.21,24 Increased urinary excretion of polyamines has now been reported in patients with various malignancies but is most pronounced in hematologic cancers.11,12,14,15,28,29 A return of polyamine excretion to normal has been postulated to reflect tumor regression in response to therapy, and the serial measurement of polyamine excretion to monitor the course of malignancy has been suggested.12,27,29 Data on the urinary excretion of polyamines in childhood cancer is, however, limited. The present report documents the measurement of polyamines in unhydrolyzed urine samples from children with acute leukemia versus a variety of other childhood cancers. Patients with cancer in remission were included in order to correlate polyamine excretion with the clinical status of the disease. Urinary polyamines in humans occur as "free" forms and as acetyl conjugates but relatively little is known about pathologic conditions causing an increase in polyamine synthesis or excretion.4,12,16 Most reports reflect total (free plus conjugated) urinary polyamines measured after acid hydrolysis.
Materials and Methods

Patients and Specimens

Three groups of patients were studied. Eighteen patients had newly diagnosed, active cancer or were in relapse. The diagnoses included hematological malignancies (acute lymphoblastic leukemia [ALL], acute myelogenous leukemia [AML], chronic myelogenous leukemia [CML], lymphoma) and solid tumors (osteosarcoma, retinoblastoma, central nervous system tumor). In ten patients, the neoplastic process (ALL) was in remission. The mean ages of the two groups of patients were 12.4 and 11.9 years, respectively. Age-matched volunteers with no diagnosed malignancy served as controls. Both sexes were equally represented in the three groups. Patients were designated in complete remission following the criteria outlined by Fembach. The patients were asymptomatic; physical examinations were normal; peripheral blood studies and bone marrow morphology were normal when they were designated to be in remission. Anything short of full remission was designated “active disease.”

Urine specimens and random (spot) urines were collected and refrigerated. Aliquot samples were frozen immediately after collection and stored without preservative at -20°C until analysis. For a comparison, approximately half of the specimens were divided and acidified with concentrated HCl to pH 1 to 2 before freezing. An aliquot of each urine specimen was taken for creatinine determination.

Analytical Methods

Cation-Exchange Analysis. Non-hydrolyzed urine specimens containing no protein were filtered through a Millipore filter (0.45 μm pore size) and 0.5 to 2.0 ml portions were lyophilized. The residue was reconstituted to one-fifth the original volume with deionized water and then centrifuged at 10,000 rpm. Forty microliters of the resulting supernatant were used for analysis. Hydrolyzed urine samples were prepared by the flushing of urine with equal volumes of concentrated HCl at 110°C for 16 hours. After acid hydrolysis, the samples were filtered and centrifuged. The supernatant was then collected and evaporated to dryness under a nitrogen-stream in a 70°C water bath. The residue was reconstituted with deionized water as described previously.

Polyamine analysis was performed on an amino acid analyzer* with fluorometer† and modified as described by Marton and Lee. Reagents: 0-pthalaldehyde 0.8 g was dissolved in 20 ml ethyl acetate : methanol (1:1). Twenty-five g of potassium hydroxide, 25 g of boric acid, and 5.8 g of potassium thiocyanate were dissolved in water and added to the OPA solution. Exactly 4.5 ml of 2-mercaptoethanol and 3.0 ml of 30 percent brij-35 were then added and the solution brought up to 1.0 liter volume.


Statistical Tests

The chi-square test for statistical differences was used for analysis of the experimental data, or when applicable, Student’s t test.

Results

The ratio of nm of urinary polyamines to mg of creatinine has been used as an expression of polyamine excretion. The use-

* Model D500, Dionex, Sunnyvale, CA.
† American Instrument Co., Silver Springs, MD.
‡ OPA, Sigma Chemical Co., St. Louis, MO.
Fullness of creatinine as a reference compound for studying urinary excretion has been established. With reference to polyamine concentration, Russell reported that serial 24 hour urine specimens from the same patient demonstrated remarkably consistent values when polyamine excretion was expressed per mg of creatinine. A comparison of putrescine:creatinine ratios was made between 24 hour urine collections and random urine samples from normal controls, and no significant difference in the values determined for the two specimens could be demonstrated. Thus, the polyamine: creatinine ratio in random urine specimens can be used as a measure of excretion. The effect of random day-to-day variation in polyamine concentration can be minimized when it is expressed per unit of urinary creatinine.

All the unhydrolyzed urine specimens examined contained detectable concentrations of putrescine and spermidine. In some instances cadaverine and spermine were also detected in the urine of control and cancer patients. Hydrolysis of the specimens resulted in significant increases in measurable spermidine and putrescine. The control value for polyamine excretion (mean ± 2SD, nmoles per mg creatinine) was 1.61 ± 1.12 for putrescine and 0.40 ± 0.20 for spermidine (table I, figure 1). The patients with active hematologic malignancies excreted significantly higher quantities of putrescine (p < 0.0025) and spermidine (p < 0.0025) as compared with the controls and the patients in remission (figure 1). Seven of eight patients with active hematologic malignancies demonstrated values for putrescine and spermidine excretion higher than either the normal control patients or those with hematologic malignancies in remission. The levels of putrescine and spermidine in the urine of patients with cancer in remission were not significantly different from those in control group (figure 1), all individual levels being within the normal range. Spermine levels were determined but are not included in this report because they demonstrated no correlation with clinical status.

Decreases in putrescine and spermidine could be demonstrated when patients entered complete remission. Although detailed serial studies were not systematically obtained, several patients had serial determinations. Generally markedly elevated values were found with active disease as opposed to normal values when patients obtained remission status.

### Table I

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Putrescine</th>
<th>Spermidine</th>
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<tbody>
<tr>
<td>Hematologic, active</td>
<td>13.67 ± 11.49</td>
<td>2.67 ± 1.61</td>
</tr>
<tr>
<td>Hematologic, remission</td>
<td>1.13 ± 0.29</td>
<td>0.46 ± 0.10</td>
</tr>
<tr>
<td>Non-hematologic</td>
<td>7.71 ± 6.92</td>
<td>6.79 ± 11.73</td>
</tr>
<tr>
<td>Control</td>
<td>1.61 ± 0.56</td>
<td>0.40 ± 0.10</td>
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</tbody>
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*p < 0.0025
Discussion

Increased serum levels and increased urinary excretion of polyamines in patients with hematologic malignancies have been reported by Russell et al.\textsuperscript{25,26,27,28,29} In 68 untreated patients, they recorded a mean urinary excretion of putrescine and spermidine two to three times that of normal controls.\textsuperscript{29,30} Moreover, increased putrescine concentrations have been recorded in the serum and urine of patients with various types of cancer.\textsuperscript{14,22,32}

Fujita et al found that total urine polyamines, especially putrescine, were increased and of diagnostic significance in patients with hematologic cancers.\textsuperscript{14} They reported a significantly greater increase in urinary putrescine in patients with hematologic cancers as compared with solid tumors. Tsuji et al reported a marked increased in urine polyamines in patients with hematologic cancers but not solid cancers.\textsuperscript{32}

A comparison of the levels of free putrescine and spermidine in random and 24 hour urine samples from children with active cancer versus age-matched controls and a group of patients in remission demonstrated significantly elevated levels in the active cancer group. These findings are similar to those reported in hydrolyzed urine of adult cancer patients.\textsuperscript{31} In this study, the highest elevations of free polyamines were observed in hematological malignancies, while other types of cancer showed varying elevations of polyamines. These findings also correspond with the previously published results of hydrolyzed urine from adult cancer patients.\textsuperscript{15,19,31,34} The high urine polyamine values observed in the patients with leukemia possibly reflect the elevated bone marrow polyamine levels reported by Rennert et al.\textsuperscript{24}

According to several studies, the remission of a neoplastic process is associated ultimately with a return of urinary polyamine excretion to the normal range.\textsuperscript{7,15,29} None of the patients in our remission group was found to have excessive polyamine excretion. The values observed in this group are perhaps the more significant in that all our cases were leukemic disorders. This result is consistent with findings previously reported by Miale et al, who related bone marrow putrescine levels to the remission status of children under treatment for leukemia and suggested a potential use for this measurement prediction of relapse.\textsuperscript{21} Rennert et al, moreover, reported cyclic variations in bone marrow levels of polyamines in leukemia patients over a period of several months, corresponding with changes in bone marrow cellularity.\textsuperscript{21,24} In a group of children with leukemia, they found the bone marrow polyamine concentrations to correlate with cellularity and percentage of malignant cells in the bone marrow. Rennert et al also reported a sharp increase in bone marrow putrescine in two patients with leukemia several weeks prior to the clinical diagnosis of relapse by bone marrow aspiration.\textsuperscript{24} Thus, Miale et al suggested that serial measurements of bone marrow polyamine concentrations in leukemic patients might be utilized in the assessment of disease status and contribute to the early detection of relapse before the full-blown manifestations become apparent.\textsuperscript{21}

It has been suggested that the increased quantities of polyamines in the extracellular fluids of cancer patients are derived primarily from tumor cells and might, therefore, serve as indicators of tumor kinetics.\textsuperscript{1,27} The increased concentrations of polyamines in cancer patients have been attributed to a combination of tumor proliferation and cell death.\textsuperscript{7,15} Increased putrescine excretion is postulated to reflect the growth fraction of neoplastic tissue, while increased spermidine and spermine are thought to be indicative of cell destruction and release of polyamines.\textsuperscript{27} Animal tumor transplant studies by Anderssen et al have shown an increase in polyamine levels and in tumor-
derived lactate dehydrogenase activity in cell-free ascites fluid during the regression of mouse Ehrlich tumor transplanted into Mongolian gerbils. Clinical observations have demonstrated two polyamine responses to successful therapy. 

In this study, random urine specimens and 24 hour urine collections were used. The urinary polyamine values measured were quantitated in relation to urine creatinine in order to correct for day-to-day variations in urine concentrations. Polyamine:creatinine values in 24 hour urine specimens from normal children did not differ significantly from values obtained from random specimens. A study of serial random urines in two adults and three children demonstrated differences in urinary polyamine:creatinine ratios of normal subjects during a 24 hour period, but the differences were far smaller than the differences between normal and cancer patients. Similar results have been reported by others who found no major diurnal variation in free or total polyamines in normal human urine. 

The use of urine polyamine excretion has been suggested as a diagnostic tool for cancer detection or as a means for assessing response to therapy. However, it should be recognized that the majority of reports have dealt with the monitoring of patients with advanced cancer, rather than with the detection of cancer. Moreover, increases in urinary polyamines are not specific for malignancies. In individual patients, the effects of certain drugs or associated medical complications must be assessed. In studies of patients with non-malignant disease receiving specific drug therapy, increased urinary polyamine excretion was found in 30 to 40 percent. Increased urinary polyamines are also commonly found in association with infectious processes and have been reported with pernicious anemia, hemolytic anemia, polymyositis, pulmonary tuberculosis, and psoriasis. 

In conclusion, the results of the present investigation imply that the measurement of free urinary polyamines may afford a simple and specific method for following disease activity and predicting relapse in childhood leukemias. Close follow-up studies on larger numbers of individual cases as well as an assessment of the role of the various non-neoplastic diseases will be needed before the full clinical evaluation can be made.

References


