Differential Excretion of Modified Nucleosides in Adult Acute Leukemia

By Debra A. Heldman, Michael R. Grever, and Ronald W. Trewyn

Excretion of modified nucleosides in urine was measured in 23 adults with acute leukemia to determine correlation of nucleoside excretion with disease activity. In addition, differences in excretion between patients with acute lymphoblastic leukemia (ALL) and patients with acute myeloid leukemia (AML) were established. Six modified nucleosides were resolved and quantitated by reversed-phase high-performance liquid chromatography (HPLC). Patients with ALL at initial diagnosis or in relapse had significantly higher concentrations of 1-methylinosine and N2,N2-dimethylguanosine in their urine compared to patients in remission (p < 0.01, p < 0.05, respectively). One patient with ALL was followed with serial nucleoside determinations over a period of 18 mo; nucleoside excretion correlated closely with disease activity. Nucleoside excretion in patients with AML did not change significantly with disease activity. Considering only those patients at initial diagnosis or in relapse, excretion of 1-methylinosine and N2,N2-dimethylguanosine was significantly higher in ALL than in AML (p < 0.01, p < 0.05, respectively). Thus, urinary excretion of 1-methylinosine and N2,N2-dimethylguanosine by adults with acute leukemia may prove to be valuable clinically in following disease activity in patients with ALL and in distinguishing patients with ALL from those with AML.

SUBSTANTIAL PROGRESS has been made in the development and use of effective multidrug chemotherapy in acute leukemia. Although 65%-80% of adults achieve complete remission with aggressive chemotherapeutic regimens, median survival in adults with either acute myeloid leukemia or acute lymphoblastic leukemia is only about 2 yr.1-3 Most failures to attain long-term survival can be attributed to bone marrow relapse. Once relapse occurs, the ability to achieve and maintain a subsequent remission is greatly reduced.4 Bone marrow transplantation provides a chance for long-term remission or cure, especially for patients in good clinical condition at the time of transplantation.5,6

Once a patient achieves complete remission, it is impossible to determine whether he is cured, in temporary remission, or about to relapse. Clinical detection of disease activity by bone marrow and peripheral blood evaluation is possible only when the tumor burden is relatively large.7 A sensitive biochemical indicator of residual or early recurrent disease would be of value in the management of these patients. Intervention with intensive reinduction chemotherapy or bone marrow transplantation when there is early indication of increased disease activity might then improve prognosis.

Elevation of urinary excretion of modified nucleosides has been demonstrated in patients with various malignancies.8-10 We have recently shown that nucleoside excretion is a marker of disease status in chronic myelogenous leukemia (CML).11 Elevated nucleoside excretion may be an early indicator of acceleration in that hematologic malignancy. Modified nucleosides are primarily components of transfer RNA (tRNA) that are excreted when the ribonucleic acid is catabolized.12,13 The mechanism of elevation of urinary excretion of these nucleosides in malignancy is obscure.

The present study examines urinary excretion of modified nucleosides from patients with ALL and AML to determine their potential value in monitoring disease activity in adult acute leukemia.

MATERIALS AND METHODS

Patient Population

Patients with AML and ALL were derived from the Ohio State University Hospital and Clinic population. Patients with AML ranged in age from 19 to 73 yr; included were 12 patients with acute myeloblastic leukemia, 2 patients with acute promyelocytic leukemia, and 3 patients with acute myelomonocytic leukemia. Complete remission in these patients was defined as ≤10% immature cells (myeloblasts plus promyelocytes) in the bone marrow aspirate, partial remission as >10% but ≤25% immature cells, and relapse as >25% immature cells. Six patients with non-T, non-B ALL who ranged in age from 15 to 67 yr were studied. Complete remission in these patients was defined as ≤5% lymphoblasts in the bone marrow and relapse as >5% lymphoblasts. Nucleoside excretion was determined in each patient at one or more times during the disease course (e.g., at diagnosis, in remission, and/or in relapse).

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**Reference Population**

The normal reference population consisted of 24 healthy hospital personnel. These included 14 males and 10 females, aged 20–50.

**Urine Specimens**

Small aliquots of early morning urine obtained from the patient and reference populations were immediately stored at −20°C without preservative until they were analyzed. Urine was collected from patients at initial diagnosis and patients in relapse prior to induction or reinduction therapy, respectively. Patients with AML in partial remission were not receiving a course of induction chemotherapy at the time urine was collected. Patients with AML in complete remission were not receiving a course of consolidation chemotherapy, but some were receiving maintenance therapy. Some patients with ALL in complete remission were on maintenance therapy. All patients had normal renal function and were free of bacterial infection at the time that the urine was collected.

**Nucleoside Determinations**

The method of Gehrke et al. was used for isolation of nucleosides from 1 ml of urine on a boronate affinity gel. Nucleosides were then resolved and quantitated by HPLC as described by Gehrke et al. and modified by Trewyn et al. A Beckman Ultrasphere-ODS (250 × 4.6 mm) column was used with a mobile phase consisting of 94% 0.01 M NH₄H₂PO₄ (pH 5.1) plus 6% methanol; the flow rate was 1–1.5 ml/min. Urinary excretion levels of 1-methylinosine (m1I), N²,N²-dimethylguanosine (m2G), 1-methyladenosine (mA), pseudouridine (Ψ), 1-methylguanosine (mG), and 2-pyridone-5-carboxamide-N'-ribofuranoside (PCNR) were determined. The minimum detection limit for each nucleoside was approximately 10 pmole. The nucleoside excretion level is expressed relative to urine creatinine. As reported previously by Gehrke et al., nucleoside levels in a random sample of urine expressed relative to the creatinine concentration of the sample are a valid representation of 24-hr excretion. Urine creatinine concentration was measured by an Astra-8 Analyzer or Creatinine Analyzer II (Beckman Instruments, Irvine, Calif.).

**Statistical Analysis**

The Wilcoxon rank sum test was used to compare groups of patients in independent samples.

**RESULTS**

Excretion of m1I for all patients evaluated is shown in Fig. 1. Normal controls had a median excretion level of 1.22 nmole/μmole creatinine. Median excretion of m1I by patients with ALL at initial diagnosis or in relapse was 4.27; median excretion of m1I by patients with AML in complete remission was 2.08. Patients with ALL had a significantly elevated excretion compared to the control group (p < 0.01). In addition, excretion of m1I by patients with ALL at initial diagnosis or in relapse was significantly greater than excretion by those in remission (p < 0.01).

Median excretion of m1I by patients with AML was 2.68 nmole/μmole creatinine at initial diagnosis or in relapse, 2.03 in partial remission, and 2.30 in complete remission (Fig. 1). There were no significant differences in excretion among the groups of patients with AML. However, patients with AML had a significant elevation of m1I excretion compared to the control group (p < 0.01). Finally, excretion of m1I by patients with ALL at initial diagnosis or in relapse was significantly higher than that observed in patients with AML at initial diagnosis or in relapse (p < 0.01).

The group of patients with ALL at initial diagnosis or in relapse had a median percentage of immature cells in the bone marrow aspirate of 71% (range 60%–90%) and a median absolute blast cell count in the peripheral blood of 2300 blasts (range 130–97,000). Likewise, patients with AML at diagnosis or...
relapse had a median of 62% (range 37%-92%) immature cells in the bone marrow aspirate and a median of 800 blasts (range 30-73,000) in the peripheral blood. There were no significant differences between these two groups of patients in terms of percentage of immature cells in the bone marrow aspirate or the absolute blast count in the peripheral blood ($p > 0.05$ for both).

A summary of mG excretion is presented in Fig. 2. Median excretion by normal controls was 1.24 nmole/μmol creatinine. Patients with ALL at diagnosis or relapse had a median excretion level of 3.45 and those in complete remission had a median level of 2.12. Patients with ALL had a significant elevation of mG excretion above the controls ($p < 0.01$). Excretion of this nucleoside by patients with ALL at initial diagnosis or in relapse was significantly higher than excretion by those in remission ($p < 0.05$).

Median mG excretion was 2.46 nmole/μmol creatinine in patients with AML at diagnosis or relapse. In patients whose disease was in partial remission, median excretion was 2.18. Patients with AML in complete remission had a median excretion of 2.66 nmole/μmol creatinine. Excretion of mG was significantly elevated in patients with AML compared to the controls ($p < 0.01$), but it did not vary significantly among the groups of patients with AML. Patients with ALL at initial diagnosis or during relapse had excretion values of mG that were significantly higher than patients with AML at initial diagnosis or relapse ($p < 0.05$).

Excretion of mA is depicted in Fig. 3. Patients with ALL at diagnosis or relapse had a median excretion level of 1.52 nmole/μmol creatinine compared to the control median of 2.29. Excretion of mA was significantly lower in these patients compared to the controls.
Elevated nucleoside excretion above control levels was also found in patients with either ALL or AML in complete remission, AML at diagnosis or in relapse, and ALL in relapse. The greatest increases occurred in mG' and PCNR, while the final mG excretion decreased to nearly normal levels.

**DISCUSSION**

The use of urinary excretion of modified nucleosides as a biochemical marker of malignancy has previously been reported for solid tumors as well as for chronic myelogenous leukemia. In the present report, we establish that adult patients with acute leukemia have significantly elevated nucleoside excretion compared to controls and that mG' and mG excretion in patients with ALL correlates with disease activity. In addition, patients with ALL at initial diagnosis or relapse are distinguishable by nucleoside excretion levels.

Certain precautions must be taken in the clinical interpretation of nucleoside excretion data. First, patients should have normal renal function, since nucleoside excretion is expressed relative to the urine creatinine. Second, patients should be free of bacterial infection. All patient blood smears were examined microscopically, and those with acute myeloid leukemia (AML) in complete remission showed 61% lymphoblasts. The next three specimens (months 11, 12, 14) were measured when the patient was in remission, and nucleoside excretion decreased to nearly normal levels, with the greatest decrease occurring in mG'. The final nucleoside levels (month 17) were obtained when the patient relapsed, prior to the reinduction therapy. At that time, nucleoside excretion was increased to levels similar to those obtained at initial diagnosis. The greatest increases occurred in mG' and PCNR.

**Table 1. Urinary Excretion of Pseudouridine, 1-methylguanosine, and PCNR**

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Pseudouridine</th>
<th>1-Methylguanosine</th>
<th>PCNR</th>
</tr>
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<tbody>
<tr>
<td>Control*</td>
<td>20.33 (14.60–29.86)**</td>
<td>0.68 (0.36–1.03)</td>
<td>0.74 (0.34–0.95)</td>
</tr>
<tr>
<td>ALL (Dx/Rel)‡</td>
<td>37.77 (26.72–122.94)</td>
<td>2.51 (1.33–6.56)</td>
<td>1.84 (1.43–2.97)</td>
</tr>
<tr>
<td>ALL (CR)¶</td>
<td>32.06 (24.10–45.19)</td>
<td>1.37 (0.77–1.94)</td>
<td>1.29 (0.93–2.97)</td>
</tr>
<tr>
<td>AML (Dx/Rel)§</td>
<td>31.14 (21.14–51.11)</td>
<td>1.34 (0.87–2.32)</td>
<td>1.37 (0.31–3.31)</td>
</tr>
<tr>
<td>AML (PR)¶</td>
<td>31.36 (24.25–34.06)</td>
<td>1.23 (1.02–1.51)</td>
<td>1.72 (1.37–1.96)</td>
</tr>
<tr>
<td>AML (CR)¶</td>
<td>33.69 (22.46–38.18)</td>
<td>1.75 (1.17–2.40)</td>
<td>1.73 (0.68–2.18)</td>
</tr>
</tbody>
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*Control values for 24 healthy adults.
‡ALL (Dx/Rel), 6 patients with acute lymphoblastic leukemia at initial diagnosis or in relapse.
¶ALL (CR), 4 patients with acute lymphoblastic leukemia in complete remission.
§AML (Dx/Rel), 16 patients with acute myeloid leukemia at initial diagnosis or in relapse.
¶AML (PR), 4 patients with acute myeloid leukemia in partial remission.
†AML (CR), 4 patients with acute myeloid leukemia in complete remission.
**Median value (range) of urinary excretion in nmole/μmole creatinine.

(p < 0.05). None of the other groups of patients (i.e., ALL in complete remission, AML at diagnosis or relapse, AML in partial remission, or AML in complete remission) showed a significant difference in mG' excretion from the controls.

Excretion data for the remaining three nucleosides quantitated by HPLC are shown in Table 1. Patients with either ALL or AML demonstrated a significant elevation of excretion above the control population (p < 0.01 for Ψ, mG', and PCNR). There were no significant differences in excretion of these three nucleosides among the different groups of patients with acute leukemia.

A longitudinal study of nucleoside excretion by a 21-yr-old male with ALL is depicted in Fig. 4. The initial nucleoside levels (month 0) were obtained at diagnosis, prior to any chemotherapy. At that time, the excretion levels of mG', PCNR, mG, and Ψ were elevated significantly above normal control values (p < 0.02 for each nucleoside). The next three sets of nucleoside excretion levels (months 1, 11, 12, 14) were measured when the patient was in remission, and nucleoside excretion decreased to nearly normal levels, with the greatest decrease occurring in mG'. The final nucleoside levels (month 17) were obtained when the patient relapsed, prior to the reinduction therapy. At that time, nucleoside excretion was increased to levels similar to those obtained at initial diagnosis. The greatest increases occurred in mG' and PCNR.

**Fig. 4.** Serial nucleoside excretion by a 21-yr-old male with ALL at diagnosis, in remission, and in relapse. The first urine specimen (month 0) was collected at the time of initial diagnosis prior to chemotherapy when the patient’s WBC was 9400 with 5% lymphoblasts and many atypical cells; bone marrow examination showed 61% lymphoblasts. The next three specimens (months 1, 11, 12, 14) were collected when the patient was in remission on maintenance therapy, with no lymphoblasts on either the peripheral blood smear or bone marrow aspirate. The last nucleoside excretion levels shown (month 17) were at the time of relapse prior to reinduction therapy when the patient’s WBC was 7300 with 31% lymphoblasts; the bone marrow showed extensive infiltration with blast cells. Excretion is calculated as nmole/mole µmole creatinine and is expressed as a percent of the mean value found in a control group of 24 healthy adults. Time is based on the number of months after initial diagnosis. Symbols used are: (Θ), pseudouridine; (Ο), 1-methyladenosine; (Δ), 2-pyridone-5-carboxamide-N'-ribofuranoside; (Δ), 1-methylinosine; (Ι), 1-methylguanosine; (□), N²,N²-dimethylguanosine.
infection, since infection (especially urinary tract infection) can cause moderate elevations in nucleoside excretion levels (unpublished data). The patients utilized in this study were free of these complicating factors.

Speer et al. reported a correlation between nucleoside excretion levels and stage of malignancy for solid tumors. Mrochek et al. reported elevation of urinary excretion of modified nucleosides from patients with adult leukemia. However, the possible relationship between level of excretion and disease status was not examined. We have recently demonstrated that patients with CML in the blastic phase have significantly higher levels of m1I, m2G, and Ψ excretion than patients in the stable phase. In the present study, we show for the first time that patients with ALL exhibit a similar relationship of m1I (p < 0.01) and m2G (p < 0.05) excretion to disease activity (Figs. 1 and 2). The potential value of serial determinations of modified nucleoside excretion in following patients with ALL is also suggested (Fig. 4). In contrast, nucleoside excretion by patients with AML does not vary with disease activity (Figs. 1, 2, and 3; Table I), and thus, it does not appear to be a marker of disease activity in these patients.

The significantly higher excretion of m1I (p < 0.01) and m2G (p < 0.05) in patients with ALL at initial diagnosis or in relapse compared to patients with AML at diagnosis or relapse demonstrated in this report (Figs. 1 and 2) may be useful in distinguishing difficult cases of acute leukemia at presentation. There were no significant differences between these two groups of patients in terms of absolute blast count in the peripheral blood or percentage of immature cells in the bone marrow aspirate; thus, the differential nucleoside excretion does not simply reflect differences in tumor load. In addition, patients with AML at initial diagnosis or in relapse had excretion levels of m1I, m2G, and Ψ that are significantly lower (p < 0.001, p < 0.01, p < 0.001, respectively) than those previously in patients with CML in the blastic phase. This may provide valuable clinical information for rapid differentiation of a patient with AML from a patient with Philadelphia-chromosome-negative CML initially presenting in a myeloblastic crisis.

In conclusion, urinary excretion of m1I and m2G in adult ALL was shown to be significantly higher in those patients at initial diagnosis or in relapse compared to those in remission. This correlation of nucleoside excretion with disease activity may have clinical value in following patients with ALL in regard to early indication of relapse or determination of response to chemotherapy. It was also shown that excretion of m1I and m2G can be used to distinguish patients with ALL at initial diagnosis or relapse from patients with AML at diagnosis or relapse and to distinguish patients with AML at diagnosis or relapse from those with CML presenting in the blastic phase. These latter findings may prove to be helpful in improving accuracy of diagnosis in difficult cases, thus allowing more appropriate treatment and early determination of prognosis.

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REFERENCES


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