Plasma and Urine Cyclic Nucleotide Levels in Patients With Acute and Chronic Leukemia


Plasma and urine levels of cyclic adenosine 3',5'-monophosphate (cAMP) and of cyclic guanosine 3',5'-monophosphate (cGMP) were measured in 35 normal subjects, in 24 patients with nonneoplastic diseases (iron deficiency anemia, peptic ulcer, and cholelithiasis), and in 50 leukemic patients. The leukemic group included patients with acute lymphoblastic leukemia, acute myelogenous leukemia, chronic lymphocytic leukemia, and chronic myelogenous leukemia. All patients were recently diagnosed and untreated, except for 5 patients with blastic transformation of chronic myelogenous leukemia who had been previously treated. There were no significant differences in plasma and urine cyclic nucleotide levels between normal subjects and patients with nonneoplastic diseases. In leukemic patients, plasma and urine cAMP levels were similar to those of normal subjects, whereas plasma and urine cGMP levels were markedly elevated. There were no significant differences in cGMP values between the various types of leukemia. After starting treatment, plasma cyclic nucleotide levels were periodically measured in 21 of the patients with acute leukemia: cGMP levels were normalized in all the 16 subjects who attained complete remission, whereas both cAMP and cGMP levels were apparently unaffected in the patients who did not respond to treatment. This suggests that plasma or urine cGMP could be used as an additional parameter to monitor the patient's response to treatment.

STUDIES on the role of cyclic nucleotides in the control of cell growth and differentiation suggest that cyclic guanosine 3',5'-monophosphate (cGMP) may be a positive and cyclic adenosine 3',5'-monophosphate (cAMP) a negative determinant in proliferative processes. As far as malignant tissues are concerned, alterations in cyclic nucleotide metabolism have been frequently found, though a consistent pattern of change has not yet been shown. Theoretically, the extracellular cyclic nucleotide concentrations could reflect alterations in their intracellular level. Urinary excretion of cGMP increased several fold in animals bearing fast-growing hepatomas or an intermediate-growing kidney adenoma. Moreover, cGMP excretion and tumor size were correlated. In these animals, however, cAMP urinary excretion was unaffected. Studies in human malignancies have not given unequivocal results. Plasma and/or urine cGMP levels significantly higher than those of normal subjects have been found by some authors in patients with various neoplastic diseases. However, Hunt et al. found urinary cGMP excretion well above the normal range in only 25 of 51 patients. Furthermore, plasma and/or urine cAMP levels have been reported to be either reduced, normal, or increased in neoplastic patients. In human leukemias, the cell patterns of cyclic nucleotides are abnormal; however, only few data on the extracellular cyclic nucleotide levels in leukemic patients have so far been reported, and in these studies there was no clear separation between treated and untreated patients. Scavennec et al. found that urinary excretion of both cAMP and cGMP was 3–5-fold higher in 19 patients with acute or chronic leukemias than in normal subjects. On the other hand, in 14 patients with acute myelogenous leukemia (AML) studied by Chawla et al., plasma and urine cGMP levels were 2–4-fold greater than the normal values, whereas cAMP levels were normal. Changes in the urinary excretion of cGMP but not of cAMP were also found by Hunt et al. in a group of 20 patients with "lymphoid and myeloid tumors."

To further characterize the cyclic nucleotide pattern in human leukemia, this study was undertaken on a large series of acute and chronic leukemia patients to ascertain if plasma cyclic nucleotide levels could be related to the type and/or stage of the disease.

MATERIALS AND METHODS

Plasma and urine cyclic nucleotide levels were determined in three groups of subjects. Normal: This group consisted of 35 healthy volunteers, 15 male and 20 female, aged 18–78 yr, mean 35.2. Nonneoplastic diseases: This group consisted of 24 untreated patients, 12 male and 12 female, aged 21–77 yr, mean 46.1. Seven patients had iron deficiency anemia, 7 peptic ulcer, and 10 cholelithiasis. Leukemia: this group consisted of 50 patients, 30 male and 20 female, aged 13–85 yr, mean 44.4. Fifteen patients had AML, 15 acute lymphoblastic leukemia (ALL), 7 chronic lymphocytic leukemia (CLL), and 13 chronic myelocytic leukemia (CML). All patients with ALL, AML, and CLL and 8 of the CML patients had been previously diagnosed and had never received therapy. The remaining 5 CML patients were in the blastic phase of the disease (CML-BC) and had been previously treated. Clinical data of leukemia patients are shown in Table I.
Plasma cyclic nucleotide levels were reevaluated in 16 leukemic patients, 9 with ALL and 7 with AML, who attained complete remission; in addition, they were also measured periodically in some patients during chemotherapy.

All patients had normal serum creatinine and blood calcium.

Twenty-four hour urine was collected into refrigerated containers. At the end of the collection, urine volumes were measured and aliquots frozen at -20°C until assayed for their content of cyclic nucleotides and creatinine.

Venous blood samples were drawn into prechilled tubes containing EDTA, to give a final concentration of 5 mM, between 08:00 and 09:00 a.m., after an overnight fast and at least 1 hr of bedrest. The samples were immediately centrifuged at 4°C, and plasma was separated and stored at -80°C until assayed.

cAMP was measured by a protein binding assay and cGMP by radioimmunoassay, using commercially available kits (Cyclic AMP Assay Kit, code TRK 432, and Cyclic GMP RIA Kit, code TRK 500, from the Radiochemical Centre, Amersham, Bucks, England). Before being assayed for cyclic nucleotides, urine was diluted 25–100 times with Tris-EDTA buffer (50 mM Tris-HCl, 4 mM EDTA, pH 7.5) while plasma was extracted with ethanol as previously described. All samples were tested in triplicate at two different dilutions at least. The intraassay and interassay coefficients of variation were less than 10% for both cAMP and cGMP. Recovery of known amounts of unlabelled cyclic nucleotides added to the samples was 95%–100%. Preincubation of plasma and urine samples for 1 hr at 37°C with cyclic nucleotide phosphodiesterase (Sigma Chemical Co., St. Louis, Mo.) reduced both cAMP and cGMP levels by more than 95%.

Serum and urine creatinine were determined by the method of Bonsnes and Taussky and blood calcium by atomic absorption spectrophotometry (Perkin-Elmer, model 400).

Statistical analysis of the data was performed by the Wilcoxon test and, when appropriate, by the Mann-Whitney U test.

RESULTS

Table 2 shows mean plasma and urine cyclic nucleotide levels in the normal subjects, patients with nonneoplastic diseases, and leukemic patients studied in the active stage of the disease. In all groups of subjects there were no significant differences in the values due to age or sex. Plasma and urine cyclic nucleotide levels of the normal subjects were within the range reported in the literature and were similar to those of the patients with nonneoplastic diseases. Also, in the leukemic patients, the plasma and urine cAMP levels did not significantly differ from those of healthy controls. In contrast, most of the leukemic patients showed markedly elevated levels of both plasma and urine cGMP levels. There were no significant differences in cGMP levels between patients with acute and chronic leukemia, although the highest values of the cyclic nucleotide were found in some patients with acute leukemia and with blastic transformation of CML. Plasma and/or urine cGMP levels were above, the normal ranges (2.72–5.92 pmole/ml for plasma and 0.12–0.70 μmole/g of creatinine for urinary excretion) in 11 of 15 ALL, 14 of 15 AML, 5 of 5 CML-BC, 87.5% of CML patients, and 71.4% of CLL patients. Both plasma and urine cAMP/cGMP molar ratios were significantly lower in leukemic than in normal subjects. More than 90% of the patients with acute leukemia and CML-BC, 87.5% of CML patients, and 71.4% of CLL patients had plasma, and/or urine cAMP/cGMP ratios below the normal ranges (2.21–7.04 and 4.71–17.70 for plasma and urine, respectively).

Plasma cyclic nucleotide levels were reevaluated in 16 patients with acute leukemia who attained complete remission. As shown in Table 3, both cGMP levels and cAMP/cGMP ratios were normalized in all these patients. From Figs. 1 and 2, which illustrate the pattern of plasma cyclic nucleotides in 7 acute leukemia patients during treatment, it is also evident that the 2 patients (G.A. and R.G.) who remained in complete remission showed over this period cGMP concentrations and cAMP/cGMP ratios always in the normal range. In the patients who failed to respond to treatment, the plasma cyclic nucleotide pattern was variable, the only constant feature being very low cAMP/cGMP molar ratios.

### Table 1. Main Hematologic Data of Leukemic Patients at Time of Diagnosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. Cases</th>
<th>Age (yr)</th>
<th>Hb (g/dl)</th>
<th>Platelets (x 10⁷/cu mm)</th>
<th>WBC (x 10⁷/cu mm)</th>
<th>Blasts (%)</th>
<th>Bone Marrow Richness</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>11</td>
<td>20</td>
<td>8.9</td>
<td>(5.3–12.6)</td>
<td>(8–167)</td>
<td>5.5</td>
<td>45</td>
</tr>
<tr>
<td>AML</td>
<td>6</td>
<td>9</td>
<td>64</td>
<td>(13–77)</td>
<td>(6.1–12.5)</td>
<td>113</td>
<td>32 (1–98)</td>
</tr>
<tr>
<td>CLL</td>
<td>3</td>
<td>4</td>
<td>55</td>
<td>(41–80)</td>
<td>(6.7–14.4)</td>
<td>128</td>
<td>38*</td>
</tr>
<tr>
<td>CML</td>
<td>6</td>
<td>2</td>
<td>53.5</td>
<td>(34–85)</td>
<td>(6.8–13.3)</td>
<td>74</td>
<td>85†</td>
</tr>
<tr>
<td>CML-BC</td>
<td>4</td>
<td>1</td>
<td>41</td>
<td>(34–55)</td>
<td>(6.4–10.9)</td>
<td>9.1</td>
<td>52</td>
</tr>
</tbody>
</table>

Data are expressed as median and range for peripheral blood and bone marrow blasts and as number of cases for bone marrow richness.

*Percentage of lymphocytes.
†Percentage of granulocytic cells.
Table 2. Plasma and Urine Cyclic Nucleotide Levels in Normal Subjects, in Patients With Nonneoplastic Diseases, and in Leukemic Patients

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Urine</th>
<th>Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Cases</td>
<td>cAMP (pmole/Day)</td>
</tr>
<tr>
<td>Normal</td>
<td>35</td>
<td>4.10 ± 0.193</td>
</tr>
<tr>
<td>Iron deficiency anemia</td>
<td>7</td>
<td>3.97 ± 0.367</td>
</tr>
<tr>
<td>Peptic ulcer</td>
<td>7</td>
<td>4.43 ± 0.432</td>
</tr>
<tr>
<td>Cholelithiasis</td>
<td>10</td>
<td>3.36 ± 0.249</td>
</tr>
<tr>
<td>ALL</td>
<td>15</td>
<td>3.44 ± 0.375</td>
</tr>
<tr>
<td>AML</td>
<td>15</td>
<td>3.30 ± 0.485</td>
</tr>
<tr>
<td>CLL</td>
<td>7</td>
<td>3.15 ± 0.592</td>
</tr>
<tr>
<td>CML</td>
<td>8</td>
<td>4.01 ± 0.458</td>
</tr>
<tr>
<td>CML-BC</td>
<td>5</td>
<td>4.21 ± 0.497</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE.
*p < 0.01 versus normal subjects (Mann-Whitney U test).

Table 3. Plasma Cyclic Nucleotide Levels in Normal Subjects and in Patients With Acute Leukemia Studied Before Treatment and After Attaining Complete Remission

<table>
<thead>
<tr>
<th></th>
<th>Normal Subjects</th>
<th>All Patients</th>
<th>AML Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>First Diagnosis</td>
<td>Complete Remission</td>
</tr>
<tr>
<td>cAMP (pmole/ml)</td>
<td>13.59 ± 0.580</td>
<td>10.63 ± 1.459</td>
<td>13.56 ± 0.842</td>
</tr>
<tr>
<td>p &lt; 0.02†</td>
<td>4.12 ± 0.137</td>
<td>10.13 ± 3.152*</td>
<td>3.61 ± 0.361</td>
</tr>
<tr>
<td>cAMP/cGMP</td>
<td>3.36 ± 0.140</td>
<td>1.69 ± 0.396*</td>
<td>3.88 ± 0.224</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE.
*p < 0.01 versus normal subjects (Mann-Whitney U test).
†Wilcoxon test.
‡p < 0.05 versus normal subjects (Mann-Whitney U test).
DISCUSSION

In agreement with previously reported data,\textsuperscript{18,19,31} the results of this study clearly demonstrate that abnormally high cGMP levels and/or markedly low cAMP/cGMP molar ratios in both plasma and urine are characteristic features of leukemic patients during the active stage of the disease. Furthermore, both cGMP levels and cAMP/cGMP ratios returned to normal values in plasma in all the AML and ALL patients who received complete remission. In untreated patients, as well as in those with acute leukemia who failed to respond to treatment, the reduced cAMP/cGMP molar ratios in plasma and urine were mainly due to the elevated cGMP levels, though in some patients they were the result of plasma and/or urine cAMP levels at the lower limit of the normal ranges. Our finding of normal plasma and urine cAMP levels in leukemic patients agrees with the data of Chawla et al.,\textsuperscript{19} whereas increased cAMP urinary excretion was found by Scavennec et al.\textsuperscript{31} in their patients. Abnormally high urinary excretion of cyclic cytidine 3',5'-monophosphate was also found in patients with acute and chronic leukemia,\textsuperscript{31} though only few data have so far been reported on the role of this cyclic nucleotide in the control of proliferative processes.\textsuperscript{35}

In other human malignancies, the plasma and urine patterns of cyclic nucleotides appear to be quite variable. Increased cGMP levels have been found in several cancer patients, though normal values have also been reported.\textsuperscript{15,20} Moreover, cAMP levels have been found to be either increased, normal, or reduced.\textsuperscript{16,21} In some hypercalcemic neoplastic patients, the ectopic production of parathyroid hormone (PTH) like peptides could account for the increased urinary cAMP excretion, whereas non-PTH-mediated hypercalcemia could explain the low urine levels of other patients.\textsuperscript{22,23,25} Alterations of the cAMP pattern in normocalcemic cancer patients are more difficult to explain, though it has been suggested that changes in plasma and urine cyclic nucleotide levels may be the result of abnormal intracellular cAMP and cGMP metabolism in neoplastic cells. The finding by Chawla et al.\textsuperscript{19} of an increased rate of cGMP entry into plasma in some cancer patients seems to support this hypothesis, though there is no direct evidence that cGMP is released from neoplastic cells themselves.

The mechanism(s) governing alterations in plasma and urine cyclic nucleotide levels in human leukemias
are still unclear. A decreased cAMP/cGMP molar ratio has been found in human leukemic leukocytes, but it was due to a relative rather than an absolute increase in the intracellular cGMP levels since, as shown by us and others, in these cells cGMP levels under basal conditions are similar to those of normal leukocytes, while cAMP levels are markedly reduced. However, the possibility cannot be excluded that there may be an abnormally increased permeability of the cell membrane to intracellular cGMP in leukemic leukocytes. In addition, it is also possible that the increased plasma and urine cGMP levels may reflect a reaction of normal tissues to the neoplasm.

The clinical studies carried out so far are insufficient to establish whether plasma or urine cGMP levels may be used as a tumor marker. However, our results suggest that in human leukemias the determination of cGMP levels and of cAMP/cGMP molar ratios in plasma and/or urine could be used as an additional parameter to monitor the patient’s response to treatment. The possible usefulness of these parameters in early diagnosis of relapse needs further investigation.

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