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Plasma Cyclic Nucleotide Levels in Acute Leukemia Patients

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To verify the clinical usefulness of extracellular cyclic nucleotide determination as a tumor marker, plasma cyclic AMP (cAMP) and cyclic GMP (cGMP) levels were measured in 70 normal subjects and 173 acute leukemia patients studied in different stages of their disease. Mean plasma cAMP levels were similar in leukemic and normal subjects, although in 48 patients in the active stage of the disease, first diagnosis, or relapse, the cAMP values were below the

normal range, and most of these patients failed to respond to chemotherapy. Plasma cGMP levels were markedly elevated in untreated patients, normalized in all patients who attained complete remission, and increased promptly to pretreatment values in patients who relapsed, suggesting that their determination may be useful to monitor the patients' response to treatment.

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CYCLIC ADENOSINE 3',5'-monophosphate (cAMP) and cyclic guanosine 3',5'-monophosphate (cGMP) have complex and perhaps opposite regulatory influences on proliferative processes, but their role in the pathogenesis of malignant transformation is still unclear.¹⁻³ Alterations in the cyclic nucleotide metabolism have often been found in neoplastic tissue, however, although a consistent pattern of changes has not emerged.⁴⁻¹⁰

Recently, more attention has been paid to the alterations in extracellular cyclic nucleotide concentrations in human malignancies due to their potential clinical usefulness as tumor markers. Plasma and urine cAMP levels are generally unchanged in normocalcemic cancer patients, however, although increased or decreased cyclic nucleotide levels have also been reported.^{9,11-21} On the other hand, increased cGMP levels and reduced cAMP/cGMP molar ratios in plasma and urine seem to be a more constant feature of cancer patients, with a relatively low frequency of false-negative values in the properly controlled studies.^{9,11,12,14,15,17-21} Furthermore, our preliminary data and those of other researchers demonstrated that changes in cGMP levels and in cAMP/cGMP molar ratios correlated with the patients' response to treatment and with the occurrence of a relapse.¹⁸⁻²¹

We report here the results of a 3-year study performed to evaluate the clinical usefulness of plasma cAMP and cGMP level determinations in monitoring acute leukemia patients during the different stages of their disease.

MATERIALS AND METHODS

Plasma cyclic nucleotide levels were measured in 70 healthy volunteers, 30 males and 40 females, aged 18 to 78 years, (mean 34.3 years) and in 173 acute leukemia patients, 103 males and 70 females, aged 12 to 91 years (mean 37.9 years). The leukemia group consisted of 113 recently diagnosed and untreated patients, 54 with acute lymphoblastic leukemia (ALL) and 59 with acute nonlymphoblastic leukemia (ANLL), and of 60 treated patients, 28 with ALL and 32 with ANLL, who were in complete remission. Plasma cyclic nucleotide levels were subsequently reevaluated in 52 of the recently diagnosed patients, 26 with ALL and 26 with ANLL, after they had attained complete remission, and in 66 patients, 30 with ALL and 36 with ANLL, who relapsed. In addition, plasma cyclic nucleotide levels were monitored monthly during chemotherapy in 18 ALL and 22 ANLL patients who eventually relapsed and in 57 patients, 28 with ALL and 29 with ANLL, who remained in complete remission over the 12- to 36-month period of the study. Cyclic nucleotide levels were also measured in both plasma and CSF of eight patients, five with ALL and three with ANLL, with an isolated leukemic involvement of CNS. The control group consisted of six ALL and five ANLL patients in complete remission without any evidence of CNS

leukemia, who had previously received prophylactic CNS therapy, and who underwent lumbar puncture as a part of the procedures to evaluate the possibility of stopping chemotherapy. All patients gave their informed consent to the lumbar puncture.

All patients had normal serum creatinine and blood calcium levels. Subjects were asked not to take any drug during the 2 days before sampling, even though in a preliminary study there was no evidence that the most common antileukemic drugs given the day before influenced plasma cyclic nucleotide levels. All subjects were on unrestricted activity and diet except for coffee, tea, alcohol, and smoking, which were not allowed on the day before sampling.

Venous blood samples were drawn between 8 and 9 AM, after an overnight fast and at least 1 hour's rest. CSF samples were obtained by lumbar puncture. The samples were added to prechilled tubes containing EDTA to give a final concentration of 5 mmol/L, immediately centrifuged at 4°C, and plasma and CSF were separated and stored at -80°C until assayed.

cAMP was measured by a protein binding assay and cGMP by radioimmunoassay (RIA) using commercially available kits (Cyclic AMP Assay Kit, Code TRK 432, and Cyclic GMP RIA Kit, Code 500; Radiochemical Centre, Amersham, England). Before being assayed for cyclic nucleotides, plasma and CSF were extracted with ethanol.²² All samples were tested in triplicate using at least two different dilutions. The intra- and interassay coefficients of variation were <10% for both cAMP and cGMP.

Statistical significance of the data was evaluated by one-way analysis of variance using Duncan's and Kramer's tests for multiple comparisons.

RESULTS

Table 1 shows mean plasma cyclic nucleotide levels in the normal subjects and in the acute leukemia patients studied in different stages of their disease. There were no age- or

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Table 1. Plasma Cyclic Nucleotide Levels in Acute Leukemia Patients Studied in Different Stages of Their Disease

Diagnosis	Cases (n)	cAMP (pmol/mL)	cGMP (pmol/mL)	cAMP/cGMP
Normal	70	14.33 ± 0.389	4.11 ± 0.093	3.56 ± 0.108
ALL first diagnosis	54	12.63 ± 0.564	11.60 ± 1.622*	1.51 ± 0.118*
ALL complete remission	54	14.70 ± 0.405	3.69 ± 0.114†	4.12 ± 0.132†
ALL relapse	30	12.21 ± 0.821	8.68 ± 1.232‡	1.80 ± 0.140§
ANLL first diagnosis	59	14.12 ± 0.581	13.64 ± 1.970*	1.52 ± 0.095*
ANLL complete remission	58	14.59 ± 0.393	3.73 ± 0.086†	4.00 ± 0.129†
ANLL relapse	36	12.03 ± 0.621	9.96 ± 2.103§	1.76 ± 0.132§

Abbreviations: ALL, acute lymphoblastic leukemia; ANLL, acute nonlymphoblastic leukemia.

Data are expressed as mean ± SE.

* $P < .01$ v normal (Kramer's test).

† $P < .01$ v first diagnosis and NS v normal (Kramer's test).

‡ $P < .05$ v complete remission and NS v first diagnosis (Kramer's test).

§ $P < .01$ v complete remission and NS v first diagnosis (Kramer's test).

sex-related differences in the cyclic nucleotide values in any group of subjects. Leukemic patients showed mean plasma cAMP levels similar to those of normal subjects, independent of the stage of the disease. Plasma cAMP levels were below the normal range (9.70 to 29.35 pmol/mL), however, in 25 recently diagnosed patients, 13 with ALL and 12 with ANLL, and in 23 patients in relapse, 11 with ALL and 12 with ANLL. On the other hand, in untreated acute leukemia patients plasma cGMP levels were significantly higher, and cAMP/cGMP molar ratios were significantly lower than those of the normal subjects. Plasma cGMP levels were above the normal range (2.42 to 5.92 pmol/mL) in 45 of 54 ALL and 48 of 59 ANLL patients, and cAMP/cGMP molar ratios were below the normal range (2.21 to 7.04) in 48 of 54 ALL and 52 of 59 ANLL patients. As previously reported,^{18,21} chemotherapy per se did not influence plasma cyclic nucleotide levels, but plasma cGMP levels and cAMP/cGMP ratios became normal in all patients who attained complete remission and remained in the normal range during all the remission period (Fig 1). In patients who relapsed, plasma cGMP levels increased and cAMP/cGMP ratios decreased to the pretreatment values (Table 1). Figure

2, which illustrates the plasma cyclic nucleotide pattern in 40 periodically monitored patients who relapsed under maintenance therapy, shows that most patients evidenced changes in cGMP levels and in cAMP/cGMP ratios before bone marrow blast invasion was observed. Table 2 shows the main hematological findings of these 40 patients during the 4 months before the relapse. There were no apparent correlations between these data and changes in cyclic nucleotide plasma levels, though platelet count was $<100,000/\mu\text{L}$ in four ANLL patients 1 month before relapse.

In patients in the active stages of the disease, first diagnosis, or relapse, plasma cGMP levels and cAMP/cGMP ratios did not correlate with the ability to respond to treatment, remission duration, or survival time. Plasma cAMP levels were below the normal range in 18 of 57 recently diagnosed and 19 of 42 relapsing patients who failed to respond to chemotherapy, however, but only in 11 of the remaining 80 responsive patients (chi-square₍₁₎ = 12.583; $P < .001$).

Patients in complete remission had CSF cyclic nucleotide levels ranging from 9.95 to 20.62 pmol/mL for cAMP and from 2.79 to 4.89 pmol/mL for cGMP. These values were in

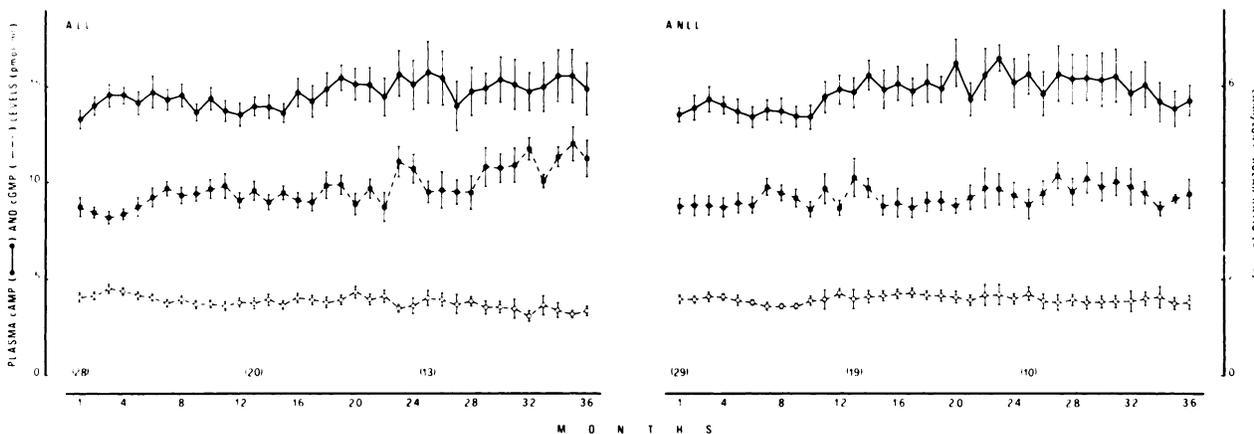


Fig 1. Plasma cyclic nucleotide levels in 57 treated acute leukemia patients who remained in complete remission. Of the patients with acute lymphoblastic leukemia (ALL), 13 were monitored for 3 years, 20 for 2 years, and 28 for 1 year; of the patients with acute nonlymphoblastic leukemia (ANLL), 10 were monitored for 3 years, 19 for 2 years, and 29 for 1 year. These numbers are shown in brackets. Data are expressed as mean ± SE.

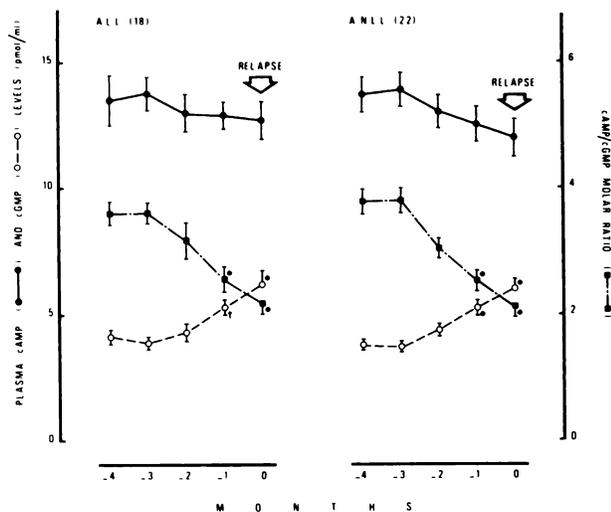


Fig 2. Plasma cyclic nucleotide levels in 18 acute lymphoblastic leukemia (ALL) and 22 acute nonlymphoblastic leukemia (ANLL) patients who relapsed under treatment. Reference time is that of relapse. Data are expressed as mean \pm SE. * $P < .01$ v findings at -4 and -3 months (Duncan's test). † $P < .05$ v findings at -4 and -3 months (Duncan's test).

general agreement with those reported in the literature for subjects without evidence of CNS disease.^{23,24} Patients with isolated CNS leukemia showed mean plasma and CSF cyclic nucleotide levels similar to those of the control subjects, with the exception of one ALL patient, with a symptomatic leukemic meningitis, who had CSF cGMP levels of 11.14 pmol/mL.

DISCUSSION

There were no significant differences in plasma cyclic nucleotide patterns between ALL and ANLL patients. Our results, however, indicate that in acute leukemias plasma cGMP levels and cAMP/cGMP molar ratios may be useful to monitor the patient's response to systemic chemotherapy and the occurrence of medullary relapse. On the other hand,

plasma and CSF cyclic nucleotide levels were similar in patients with and without CNS leukemia, indicating that their determination does not have a role in the early diagnosis of CNS involvement.

Elevations in cGMP levels and reductions in cAMP/cGMP molar ratios in plasma and urine often occur in hematological malignancies^{9,12,14,15,20,21} and in solid tumors,^{11,15,17-19,21} although the frequency of normal values varies in the different studies. In our patients in the active stages of leukemia, first diagnosis, or relapse, the frequency of false-negative results was low, $<15\%$, when either cGMP levels or cAMP/cGMP ratios were considered. The reduced cAMP/cGMP ratios generally reflected an increase in plasma cGMP levels; in $\sim 20\%$ of the patients, however, they were due to plasma cAMP levels below the normal range. In agreement with previously reported data indicating that decreased extracellular cAMP levels are not a frequent feature of normocalcemic cancer patients,^{9,11-21} in this study only 48 patients had markedly reduced plasma cAMP levels; this finding, however, was an unfavorable prognostic indicator since most of these patients failed to respond to chemotherapy.

Because changes in extracellular cyclic nucleotide levels occur in different malignant diseases, they are not specific for leukemias and the mechanisms governing their development are still unclear. Extracellular cyclic nucleotide concentrations may reflect changes in their intracellular metabolism, since both adenylate cyclase-cAMP and guanylate cyclase-cGMP systems are unbalanced in human leukemic leukocytes.⁸⁻¹⁰ Certainly, an enhanced egress of cGMP from cells¹⁵ could account for the increased plasma cyclic nucleotide levels we found in the active stages of acute leukemias, though no direct evidence shows that cGMP is released from leukemic leukocytes rather than from normal tissues as a reaction to the malignant process. On the other hand, it seems unlikely that a reduction in cAMP levels in leukemic cells may in itself influence the plasma cyclic nucleotide concentrations, due to the many factors known to affect extracellular cAMP levels.

Studies in cancer-bearing animals have demonstrated that

Table 2. Main Hematological Data in 18 ALL and 22 ANLL Patients Who Relapsed Under Maintenance Therapy

Months Before Relapse	ALL					ANLL				
	Peripheral Blood			Bone Marrow		Peripheral Blood			Bone Marrow	
	Hb (g/dL)	WBC ($\times 10^9/L$)	Platelets ($\times 10^9/L$)	Blasts (%)	Cellularity* ↓ N ↑	Hb (g/dL)	WBC ($\times 10^9/L$)	Platelets ($\times 10^9/L$)	Blasts (%)	Cellularity* ↓ N ↑
-4	13.9 (9.4-16.1)	5.0 (3.0-12.6)	258 (110-450)	3.00 (1.25-6.00)	6 7 5	12.9 (10.1-15.5)	4.6 (1.8-10.5)	245 (89-608)	4.25 (1.25-6.75)	6 15 1
-3	13.9 (9.1-15.7)	4.5 (1.8-26.0)	259 (91-609)			12.8 (10.9-15.4)	4.9 (2.9-9.1)	258 (104-817)		
-2	13.8 (10.5-16.6)	4.7 (3.1-15.8)	240 (105-731)	2.75 (0.25-7.25)	6 8 4	13.0 (9.8-15.5)	4.6 (1.7-9.4)	275 (95-658)	4.75 (2.00-11.50)	8 9 5
-1	14.1 (9.1-16.8)	4.1 (2.2-15.0)	204 (125-429)			13.0 (10.7-16.0)	3.7 (1.8-10.9)	206 (16-552)		
0	13.5 (10.3-16.9)	5.4 (1.2-21.7)	134 (6-350)	38.0 (10.0-67.0)	6 5 7	12.4 (6.5-15.5)	3.2 (0.8-17.0)	152 (12-408)	26.0 (10.0-89.0)	10 7 5

Data are expressed as median and range for peripheral blood and bone marrow blasts.

*↓, Hypocellular, N, normocellular, ↑ hypercellular; the numbers under this heading refer to the number of marrows with the indicated level of cellularity.

extracellular cGMP levels correlate with tumor size.^{25,26} A similar correlation could not be determined in our patients since the number of blasts in peripheral blood as well as the bone marrow richness are only indirect indicators of the leukemic burden. The finding that plasma cGMP levels and cAMP/cGMP molar ratios became normal in all patients who attained complete remission and changed precociously in patients who relapsed suggests, however, that these parameters behave as tumor markers. On the other hand, alterations in extracellular cyclic nucleotide levels have also been reported in some patients with benign tumors and with non-neoplastic diseases,^{13,17,18,22,27} indicating that the finding of increased cGMP levels and/or reduced cAMP/cGMP ratios cannot be used in a previously undiagnosed subject as

an unequivocal indication of the existence of a malignant process.

In conclusion, evidence shows that plasma cyclic nucleotide determination may be of clinical relevance as an additional parameter to monitor leukemic disease activity and may also suggest a more aggressive therapeutic regimen in recently diagnosed or relapsing patients with low plasma cAMP levels in view of their negative prognostic significance.

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