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Relationship between plasma adriamycin levels and the outcome of remission induction therapy for acute nonlymphocytic leukemia

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Summary. *Plasma adriamycin and adriamycinol levels were masured in 45 patients with acute nonlymphocytic leukemia 3 h after the drug was administered. A wide range of levels was found. Plasma levels increased after the administration of each of three daily doses of the drug. High plasma levels were associated with both death during remission induction therapy and, for patients who entered remission, long remissions.*

Introduction

The efficacy of remission induction therapy for acute nonlymphocytic leukemia (ANLL) has gradually improved, with remission rates generally being reported to range from 55% to 75% [9, 10, 13, 15]. Patients who do not enter remission are distributed between those whose leukemia is resistant to the regimen administered and those individuals who die during remission induction therapy [8–10]. As part of our studies of the factors which affect response to remission induction therapy, we have measured plasma adriamycin levels in 45 patients receiving cytosine arabinoside/adriamycin remission induction therapy. In these patients we found a wide range of plasma levels 3 h after the adriamycin was administered. High plasma adriamycin levels 3 h after the first dose of drug was administered were associated with death during remission induction therapy and, for those who survived induction therapy and entered remission, long remissions.

These observations may provide the basis for altering remission induction regimens while they are being administered to reduce induction morbidity and mortality.

Methods

The criteria of the French-American-British working party were used to diagnose acute nonlymphocytic leukemia (ANLL) [3]. The patients were treated at one of the Leukemia Study Group institutions either at the time of initial diagnosis or at the time of first relapse with a remission induction regimen consisting of cytosine arabinoside (araC) 100 mg/m^2 per day for 10 days, administered by continuous IV infusion, together with adriamycin (adr) administered on days 1, 2, and 3 by IV infusion over 15–30 min. The dose of adr was 30 mg/m^2 per day for patients $\leq$ 60 years of age and 20 mg/m^2 per day for patients $>$ 60 years. Patients received cotrimoxazole, two regular strength tablets twice daily, from the start of therapy until the granulocyte count exceeded 500/μl [11]. Patients were ineligible for this induction regimen if they had received araC/anthracycline antibiotic therapy within the 3 months prior to relapse of if their serum bilirubin was $>$ 3 mg/dl.

Complete remissions were defined by the criteria proposed by Cancer and Acute Leukemia Group B [12]. In brief, this meant a reduction in the proportion of myeloblasts or leukemic cells to $<$ 5% of the marrow cells, together with a return of hematopoiesis and peripheral blood counts to normal. Clinical drug-sensitive disease was deemed to be present if the patient entered complete remission (CR). Patients who did not enter remission were classified as having failed induction therapy because of resistant disease (RD) or for 'other' reasons [6, 8]. For drug-resistant disease to be present, the patient had to survive a minimum of 17 days after the start of therapy, and the day 17 bone marrow examination had to demonstrate persistent leukemia or, if marrow hypocellularity had been induced by therapy, the patient had to survive long enough for leukemic cells to repopulate the marrow. The 'other failure' category included patients who expired before day 17 of therapy and patients who expired after day 17 with a severely hypocellular bone marrow. A single patient whose bone marrow remained severely hypocellular for 90+ days after the initiation of chemotherapy was also included in this category. The other failure patients share in common the fact that they

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would not have benefited from more aggressive therapy, since none expired as a result of leukemic complications. Rather these patients might have benefited from less agressive therapy.

A detailed survey of the medications that each patient was receiving immediately before and during adr administration was carried out to discern whether the administration of any medication altered the plasma levels of adr or its metabolities.

Measurement of plasma adr and metabolite levels. Blood samples were drawn into heparinized tubes immediately before and at 3 h after each dose of adr was administered. The 3-h time point was selected since on the basis of our own studies and of prior studies in the literature [1, 2, 14], this represents a point where a small difference in sample acquisition time does not have a major effect on plasma levels. Moreover, it is close to the inflection of the plasma decay curve and is under the influence of the penultimate and ultimate phases of pharmacokinetics, which together contribute more than 70% of the area under the curve.

The blood samples were centrifuged at 4° C and the plasma removed and frozen. Specimens were shipped in a frozen state to Roswell Park Memorial Institute where the analyses were performed. The samples were analyzed as described in detail by Bolanowska et al. [4]. Briefly, a thawed plasma sample was sonicated and a 0.5-ml aliquot was vigorously mixed with 1 ml 0.6 *N* hydrochloric acid in absolute ethanol. The samples were kept at 0–4° C for 3–24 h, then centrifuged at 40,000 *g* for 20 min. Then 50–150 µl of clear supernatant were injected onto an HPLC column containing µ-bondapak-phenyl packing with particle size 10 µm (Waters Assoc. Inc., Milford, MA). The mobile phase consisted of 27% acetonitrile in 0.1 *M* ammonium formate buffer with pH 4.0; the flow rate was 2 ml/min. Detection of anthracycline was accomplished with a spectrophotofluorometer, Model 650-10S (Perkin-Elmer, Norwalk, Conn), using excitation at 470 nm and emission at 585 nm. Retention times and areas of the peaks were recorded by the data station Sigma-10 (Perkin-Elmer, Norwalk, Conn.). Quantitation was effected by reference to standard curves for adr and adriamycinol (adrol) in the 10–1,000 ng/ml concentration range. Standards were run daily. An impulse-to-noise ratio of 3 : 1 was achieved at 0.3 ng adr and the limit of quantitation was 3 ng/ml plasma.

Statistical analyses. For the purpose of this study both the previously untreated patients and the first relapsed patients were analyzed together to ensure more meaningful statistical results.

Since the distribution of the plasma adr levels was unknown, the distribution-free Mann-Whitney test was used to compare the plasma adr levels in patients with different responses to the remission induction therapy.

The correlations between plasma adr levels on days 1, 2, and 3, or between plasma levels and remission duration are expressed in terms of Pearson correlation coefficients. In addition, the Kendall rank correlation was used to double-check the significance of the correlation.

For some analyses the plasma adr levels were normalized by dividing the plasma level by the dose of adr, and then multiplying the result by 30 mg/m^2.

Multivariate linear analysis was used to determine whether SGOT and bilirubin together with age would permit recognition of those patients who would have high plasma adr levels.

Results

Individual variation in plasma levels of adr

Figure 1 provides information regarding the daily 3-h plasma adr levels produced by the 20-mg/m^2 and 30-mg/m^2 dosages of adr. The mean plasma levels produced by the 20 mg/m^2 dosage were two-thirds those produced by the 30-mg/m^2 dosage. The 30-mg/m^2 dosage produced statistically significantly higher plasma adr levels on days 1 and 2 than did the 20-mg/m^2 dose level. Each daily dose resulted in progressively higher 3-h plasma adr levels in both groups of patients.

The data presented in Fig. 2a–c demonstrate that the progressive rise in the daily plasma adr levels was a phenomenon which occurred for individual patients and was not merely a population effect reflecting variation in the method of measurement. For example, the correlation coefficient for adr levels on days 1 and 2 was 0.8063 ($P = < 0.001$). These same patterns were noted when the adr levels were normalized for dose levels administered.

Figure 3 compares plasma adr levels produced by the administration of adr to 32 patients who had not been treated with an anthracycline antibiotic in the past versus 12 patients who had received adr in the past and to a single patient who had received daunomycin in the past. The values were normalized to a uniform dose of adr. No differences were noted between these two patient groups.

Various clinical parameters were studied to determine their relationship to the actual and normalized plasma adr and adr + adrol levels on days 1, 2, and 3. The following factors were not related to plasma adr levels: age, body temperature, presence or absence of infection, leukocyte count, number of circulating leukemic cells, hematocrit, platelet count, administration of other drugs, SGOT, LDH, bilirubin, FAB type of leukemia, and treatment at initial diagnosis or at relapse. Multivariate analyses of these patient characteristics failed to demonstrate a combination of variables which were useful for predicting plasma drug levels.

Relationship of plasma adr levels to outcome of therapy

The clinical aspects of the studies presented here are summarized in Table 1. Thirty-two patients were studied at the

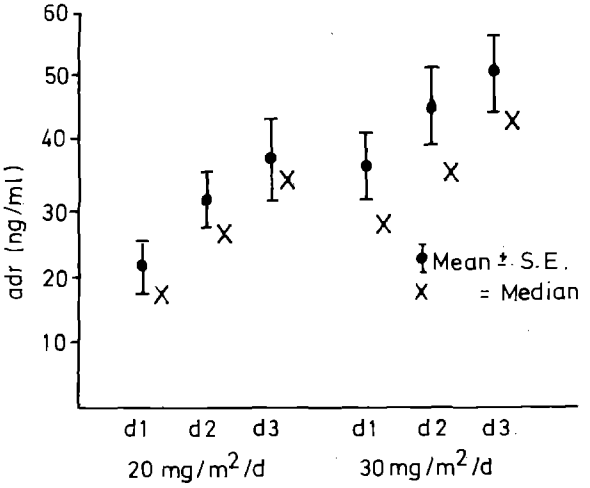
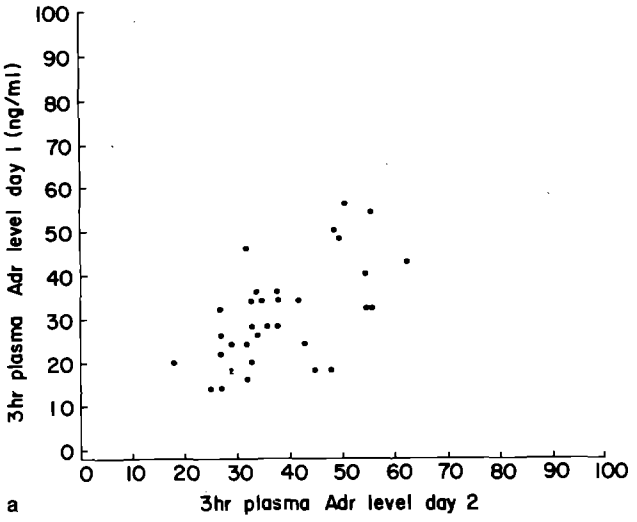
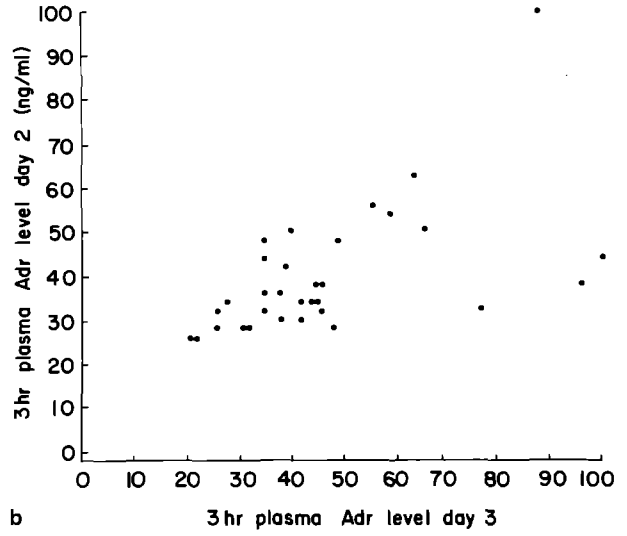


Fig. 1. Relationship between dose of adr administered and daily plasma adr levels 3 h after adr administration. *Points* and *bars* indicate means ± SE; ×, median





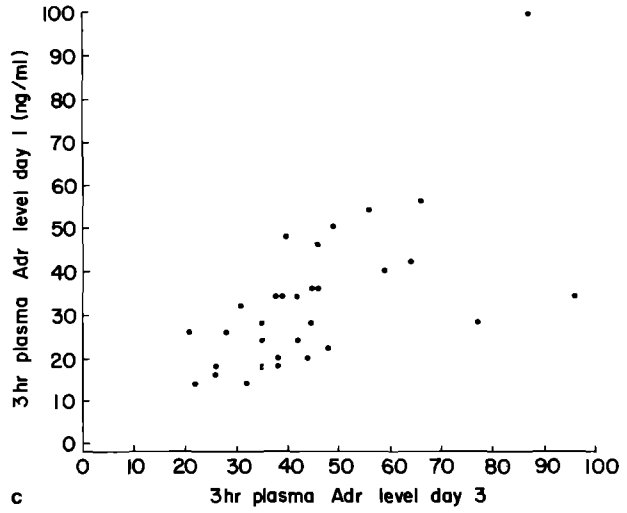
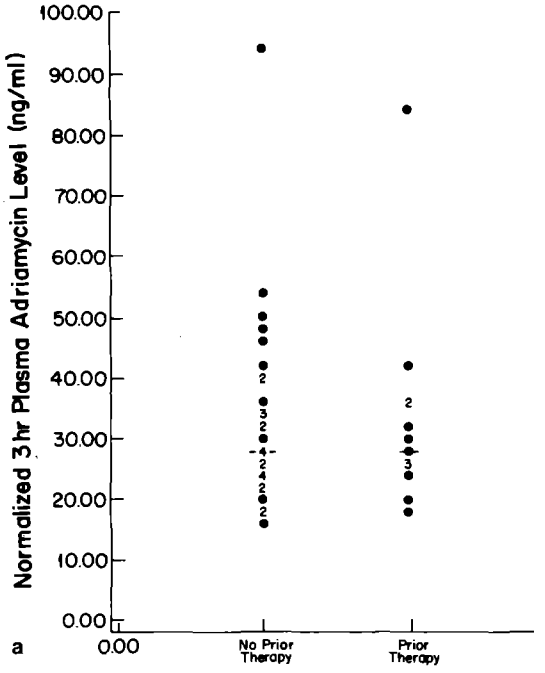
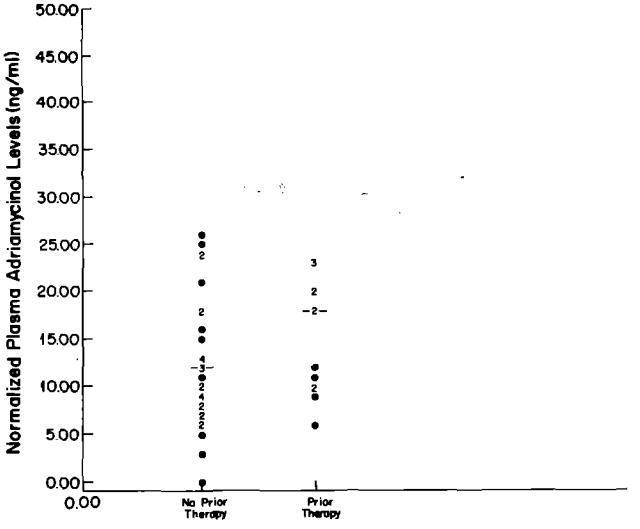


Fig. 2a–c. Relationship between daily 3-h plasma adr levels of individual patients. **a** 3-h level on day 1 vs 3-h level on day 2: $r = 0.8063$, $P = 0.001$; **b** 3-h level on day 2 vs 3-h level on day 3; $r = 0.4984$, $P = 0.003$; **c** 3-h level on day 1 vs 3-h level on day 3: $r = 0.5408$, $P = 0.001$. Each *point* on the graph represents the 3-h plasma adr level for an individual patient on the indicated days. The data were not normalized





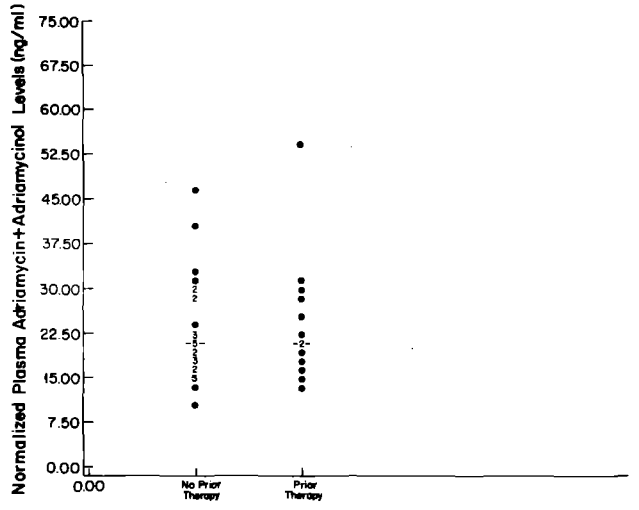


Fig. 3a–c. Comparison of day 1 normalized 3-h plasma adr and/or adrol levels for patients who had or had not received therapy with adr in the past. **a** adr level; **b** adrol level; **c** adr + adrol level. Figures represent numbers of values coincident with particular points

time of initial diagnosis, while 13 patients were in first relapse. The complete remission rate was 66% for previously untreated patients and 62% for first-relapse patients, with an overall CR rate of 64%. By definition no CR patient died during induction therapy. The range of survival for the CR patients is 40–615+ days from the start of therapy. Five of the eight patients who had drug-resistant disease have expired with mean ± SE and median times to death of 192.4 ± 57 and 163 days, respectively. Three patients have been lost to follow-up. There were eight other failures. Seven expired from infection and/or bleeding at a mean ± SE and median of 19.3 ± 4.7 and 22 days, respectively from the initiation of therapy to death. Seven patients died between day 1 and day 33 of therapy. The eighth patient was alive 3 months after induction therapy with a severely hypocellular bone marrow.

Table 1. Relationship between outcome of remission induction therapy and dosage of adriamycin administered

	Dose of adr	CR	RD	'Other' failures
Patients at initial diagnosis	20 mg/m^2	6	0	1
	30 mg/m^2	15	5	5
Patients treated at 1st relapse	20 mg/m^2	3	0	1
	30 mg/m^2	5	3	1
All 20 mg/m^2 patients		9	0	2
All 30 mg/m^2 patients		20	8	5

Figure 4a and b presents the relationship between the actual (nonnormalized) day 1 plasma adr and (adr + adrol) ÷ 2 and the outcome of remission induction therapy. The drug levels within each outcome group varied over a wide range. The range of values for CR and RD patients were similar, while the values in other failures showed the greatest variability. Four of the eight patients classified as other failures had plasma adr and (adr + adrol) ÷ 2 levels in excess of those found in the majority of the surviving patients (be they CR or RD patients). These differences approached a level of statistical significance, at 0.08 for comparison of adr + adrol levels of CR patients and other failures. Infection was the primary cause of death for seven of the eight other failures, while one patient died because of hepatic failure. Associated causes of death were renal failure in two patients, respiratory failure in one patient, and congestive heart failure in two patients.

Given the higher plasma adr + adrol levels in patients who proved to be other failures, a variety of clinical parameters were evaluated to determine whether they were also associated with other failure type (Table 2). Patients who were other failures tended to be older than CR or RD patients and tended to have higher bilirubin and SGOT levels as well. None of these differences was statistically significant. Renal function was also comparable for all groups. Linear regression analysis

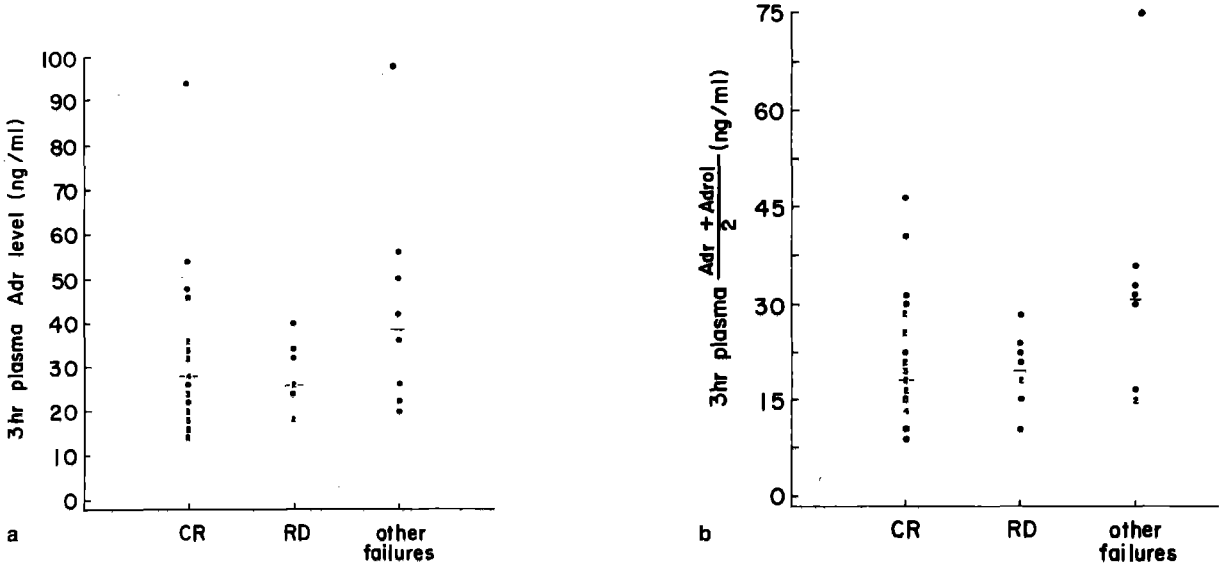


Fig. 4a and b. Relationship between the 3-h adr and adr + adrol levels on day 1 of therapy and the outcome of remission inducation terapy. **a** adr levels; **b** adr + adrol levels

Table 2. Comparison of clinical measurements in patients in whom adr levels were measured

	CR patients			RD patients			'Other' failures		
	Mean ± SE	Median	Range	Mean ± SE	Median	Range	Mean ± SE	Median	Range
Age	47 ± 3	53	15– 71	39 ± 7	30	19– 70	54 ± 6	60	18– 6
Bilirubin	0.7 ± 0.1	0.7	0– 2	1 ± 0.1	1.1	0– 1	1.1 ± 0.3	0.8	0– 3
SGOT	46 ± 6	33	17– 170	51 ± 13	34	20–138	100 ± 46	32	11– 3
LDH	413 ± 66	355	122–1,225	439 ± 101	412	158–939	525 ± 156	295	270–12
AIK p'tase	101 ± 15	79	39– 405	110 ± 17	98	59–223	184 ± 67	105	45– 6
Creatinine	1.04 ± 0.06	1	1– 2	0.9 ± 0.1	0.9	1	1.04 ± 0.2	1	1– 2

combining SGOT, bilirubin, and age did not permit recognition of those patients who would have high plasma adr levels and expire during therapy.

There was a significant correlation between the 3-h plasma adr and adr + adrol levels on day 1 and remission duration. For all patients studied the correlation coefficient between adr level and remission duration was 0.561 ($P = 0.002$) and that between adr + adrol and remission duration was 0.435 ($P = 0.018$). For previously untreated patients the correlation coefficient between adr or adr + adrol values and remission duration was 0.558 ($P = 0.009$) and 0.451 ($P = 0.04$), respectively. For first-relapse patients the correlation coefficients were 0.457 ($P = 0.255$) and 0.4802 ($P = 0.228$) for adr and adr + adrol, respectively. The lack of statistical significance for the latter correlations may be related to the small number of patients with relapsed disease who were studied ($n = 8$). The plasma adr and adr + adrol levels on days 2 and 3 of therapy were not significantly correlated with remission duration.

Discussion

Administration of the same dose of adr to patients with ANLL results in a wide range of 3-h plasma adr and adrol levels. None of the preclinical variables studied was predictive of the levels achieved. It should be remembered, however, that as described in *Methods*, patients whose bilirubin was 3.0 mg% or higher were excluded from therapy. Reduction in the adr dosage to 20 mg/m^2 in patients > 60 years old resulted in the production of 3-h plasma adr levels which were two-thirds those achieved wth the 30-mg/m^2 dosage. The administration of adr on 3 successive days resulted in progressively higher 3-h adr levels.

A relationship was found between death during induction and plasma adr and adrol levels measured 3 h after administration of the first dose of drug. Four of the eight other failures had plasma adr + adrol levels which exceeded those of almost all of the surviving patients, whether they entered CR or proved to have RD. It is interesting that the plasma adr level was highly correlated with induction mortality in patients who received a combination chemotherapy regimen which included a 10-day infusion of araC. It is known that adr produces significant gastrointestinal toxicity. A breakdown of mucosal barriers with bleeding and hematogenous seeding of enteric bacteria might be responsible for the association of death during induction with high plasma adr levels.

The wide range of plasma adr levels achieved with administration of identical dosages suggests that modulation of the magnitude of the second and third doses based on the day-1 plasma level or on levels produced by a pretherapy 'test dose' would be possible. This approach might decrease the morbidity and mortality associated with induction therapy, since it would permit a reduction in adr dosage for those patients in whom the standard dosage would produce inordinately high plasma levels. Similarly a dose ascalation for patients in whom the conventional dosage produces low plasma levels might result in fewer treatment failures due to resistant disease and longer remission durations.

The measurement of plasma adr levels achieved during remission consolidation chemotherapy may also be of relevance, since high levels may be associated with inordinate toxicity and low levels with inadequate antileukemic effects. We have in the past reported observations which suggested that, in some patients, repeated courses of adr result in progressively lower plasma drug levels [5]. If confirmed, these observations could explain the phenomenon of leukemic relapse with leukemic cells which are sensitive to the drugs being administered to the patient [7]. Therefore, in the setting of consolidation therapy, modification of doses administered on the basis of measured plasma drug levels might also be useful.

The studies described here demonstrated a modest but statistically significant relationship between the 3-h plasma adr level on day 1 of therapy and remission duration. This correlation is most probably due to the relationship between the amount of drug delivered to the target cells and the number of leukemic cells killed. The fact that the correlation coefficient is in the range of 0.5 is to be expected, since the plasma adr level is undoubtedly only one of several factors which determine the duration of remission for patients who are treated with combination chemotherapy.

The studies reported here illustrate the clinical relevance of pharmacokinetic studies. More detailed pharmacokinetic studies are likely to provide still more useful information.

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