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Growth Response of Residual Leukemia after Initial Drug Therapy[1]

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ABSTRACT

Clinical trials with laboratory correlates were conducted in patients with acute leukemia to determine if relationships exist between drug dose and growth of surviving leukemia cells. The therapeutic design was based on findings in the leukemic rat that relate the initial dose of drug and tumor kill to the magnitude of residual tumor proliferation and sensitivity to a second drug. Patients with acute myelocytic leukemia received cytarabine, either 2 or 6 g/m²/72 h by continuous infusion. The presence and magnitude of change between initial and residual tumor after treatment, as measured by change in labeling indices, depended on the "priming" dose of drug. The amount of perturbation correlated with clinical response to cytarabine given at the time of induced proliferation. With results which parallel the rat data, the direct relationship of initial drug dose and proliferation of residual tumor is demonstrated in humans, and lends support to the design of our clinical trials of timed sequential therapy.

INTRODUCTION

Timed sequential chemotherapy of hematopoietic malignancy is based on temporally predictable drug-induced growth of the tumor cell population remaining after initial drug administration and the S-phase specific mechanism of action of ara-C[3] given at that time (1–10). Studies for a rat model of AML have correlated expanding tumor volume with a declining tumor growth fraction (2–4). Reduction in the tumor mass by initial drug effects a dose-related increase in proliferation of the remaining malignant cohort.

This dose-related stimulation and predictable recruitment of residual tumor to DNA synthesis increases the action of cell cycle-active agents given at that time. Enhanced ara-C cytotoxicity has been demonstrated by increased survival in rats bearing late stage AML and cures of rats in remission with minimal residual disease, but only when ara-C is given in a timed sequence coinciding with the predicted peak in residual tumor proliferation. Based on these studies in the rat model which related initial dose of drug and tumor kill with antitumor effect, we have designed and conducted trials in patients with AML which define a similar summation of factors. In humans as in the rat, this constellation combines drug dose, tumor mass, magnitude of proliferation, and drug timing with clinical response.

MATERIALS AND METHODS

Patients and Therapy. From January 1975 through January 1979, and May 1983 through March 1984, a toal of 84 newly diagnosed, nonpreviously treated adults with the diagnosis of AML (myeloid, myelomonocytic, and monocytic) were studied during intensive timed sequential induction chemotherapy with ara-C and daunorubicin (9, 10). All patients received this therapy as part of clinical investigations initiated only after approval of the Johns Hopkins Human Investigations Committee in accord with assurance approved by the United States Department of Health and Human Services, and following full informed consent to participate in active investigative therapy. Thirty-seven patients treated with a single cycle of AcDAc have been previously reported with respect to clinical outcome following therapy (9).

Of these 84 nonpreviously treated patients, 66 (January 1975 to January 1979) received an initial 72-h continuous infusion of ara-C at a total dose of 2 g/m²/72 h, and 18 (May 1983 to March 1984) received an initial 72-h continuous infusion of ara-C at a total dose of 6 g/m²/72 h. All patients concomitantly received daunorubicin, 45 mg/m²/day, for 3 days as part of initial drug therapy, and subsequently received a second 2-g/m²/72 h continuous infusion of ara-C on day 10 following initial institution of AcD on day 1. All patients were evaluated for evidence of CTC or NR between days 14 and 16 by bone marrow aspirates and biopsies. Complete remission, defined by Acute Leukemia Group B criteria, was ultimately achieved in all patients with CTC who did not die of toxicity. Those patients treated with an initial ara-C dose of 2 g/m²/72 h had a median age of 54 years (range, 16–74), while those patients who received an initial ara-C dose of 6 g/m²/72 h had a median age of 43 years (range, 20–65). All patients admitted for diagnosis and therapy were studied and were categorized retrospectively only on the basis of survival through day 16 of therapy, at which time CTC and thus response to induction therapy could be evaluated. Of the 66 consecutively evaluable patients given AcDAc (2 g/m²/72 h), 37 (56%) achieved CTC and 30 (46%) achieved CR, while 16 (89%) of the 18 consecutively treated patients receiving 6 g/m²/h AcDAc achieved CTC and 12 (67%) achieved a CR.

Bone Marrow Cell. Marrow cells were obtained from all patients by routine needle aspiration, collected in RPMI 1640. To ensure that marrow cells were free from contamination by peripheral blood blasts, marrow spicules were isolated and cells were monodispersed by drawing through a 25-gauge needle. Each cell suspension was prepared and studied individually. Cells were obtained for study prior to therapy and again, concomitant with serum collection, prior to the second ara-C infusion.

Specific Sera Collection. Prior to therapy and prior to the second ara-C infusion, 20 ml of whole blood were collected from each patient and allowed to clot. Sera were separated sterilely and stored immediately at −70°C until use.

[³H]dThd LI Determinations. Aliquots of aspirated bone marrow cells from patients with 75% leukemia marrow blasts were obtained prior to initial AcD therapy and again on day 10 prior to the second ara-C infusion to determine drug-induced changes in tumor proliferation. Of the 66 patients who received 2 g/m² ara-C initially, 50 had residual bone marrow leukemic cells on day 10 for determination of LI; of the 18 patients given 6 g/m² ara-C initially, 14 had post-AcD leukemic cells adequate for study. Aliquots of monodispersed marrow cells were incubated with 15% autologous serum obtained at the time of that marrow aspiration and [³H]dThd (0.1 μCi/ml; specific activity, 2 Ci/mmol) for 75 min at 37°C in a humidified atmosphere containing 5% CO_2. Following incubation, cells were washed carefully 3 times in phosphate-buffered saline and lightly cytocentrifuged at 1000 *g* for 5 min onto slides coated with gelatin. Autoradiographs were prepared with Kodak NTB-2 photographic emulsion exposed for 21 days, developed, and stained with Giemsa. The [³H]dThd LI of leukemic blasts was determined by counting the number of cells per 1000 blasts that contain >5 grains overlying the nucleus. Background labeling is estimated by the number of grains present in a cell-free area equivalent to the area of the blast nucleus and was negligible (≤1 grain), while labeled cells contained ≥25 grains. Results are expressed as percentage of labeled leukemia blasts and the change in LI from pretreatment to post-

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[3] The abbreviations used are: ara-C, cytarabine; AML, acute myelogenous leukemia; AcDAc, timed sequential chemotherapy with ara-C and daunorubicin; CTC, complete tumor clearance; CR, complete remission; NR, no response; [³H]dThd, tritiated thymidine; LI, labeling index; ΔLI, ratio of change in LI from day 0 to day 10 of therapy.

AcD values was compared (ΔLI). The standard error for this method is ±1%.

Statistical Methods. Comparisons of drug-induced changes in residual tumor proliferation in patients receiving 2 g/m² ara-C and patients receiving 6 g/m² ara-C initial infusions were analyzed using Student's *t* test and Fisher's exact *t* test. The relationship between detected drug-induced changes and clinical outcome (CTC, CR, and NR) was analyzed by Fisher's exact *t* test.

RESULTS

An initial dose of 2 g/m²/72 h AcD produced a mean 1.21-fold increase in post-AcD tumor LI over pretreatment values (ΔLI, Fig. 1; Table 1). The relationship between initial ara-C dose and drug-induced cell growth kinetic perturbation was established by achieving a greater tumor LI change in those patients treated initially with 6 g/m²/72 h AcD (ΔLI, 3.24; $P < 0.00005$). These findings were valid for the subpopulation with CTC, as well as for all patients. The proportion of patients having marked changes in [³H]dThd LI following initial drug treatment (ΔLI ≥ 1.2) was significantly greater for patients receiving the 6-g/m²/72 h ara-C schedule.

Of the 50 patients receiving initial 2 g AcD/m²/72 h, 19 had pre-AcD LI < 10%, while 11 of the 14 patients receiving 6 g/m²/72 h AcD had a relatively low pretreatment LI (Fig. 1). When only these subgroups were examined for dose-related magnitude of residual tumor proliferation, the patients receiving 2 g/m²/72 h AcD had a mean ΔLI of 1.42, whereas the degree of induced growth for patients receiving 6 g/m²/72 h AcD was significantly higher (mean ΔLI, 3.53; $P < 0.008$). The CTC subpopulations of these patients with relatively low pretreatment leukemic cell growth behaved similarly, with the mean ΔLI for 11 such patients receiving 2 g/m²/72 h AcD being 1.69 and the mean ΔLI for the 10 CTC patients receiving 6 g/m²/72 h AcD being 3.99 ($P < 0.03$).

The presence and magnitude of drug-perturbed cell growth related to clinical outcome. Of the 38 patients who achieved CTC following timed sequential induction therapy with AcDAc and who had evaluable pretreatment and day 10 tumor cell [³H]dThd LI, 25 (66%) demonstrated drug-stimulated tumor growth (ΔLI ≥ 1.2), while only 9 of 26 (35%) similarly evaluable NR patients achieved comparable growth enhancement ($P < 0.015$). Comparison of the 28 CR patients with the 26 NR patients yielded similar differences in malignant cell DNA synthesis. When patients receiving the 2-g/m²/72 h AcD dose were analyzed separately, 15 of 26 (58%) CTC patients achieved ΔLI ≥ 1.2 (mean Δ 1.37) while only 8 of 24 (33%) NR patients had such LI increase (mean Δ 1.04), suggesting a difference in growth perturbation ($P = 0.07$) that might relate to clinical outcome.

Table 1 *Relationship of drug dose and drug-induced perturbation of leukemic growth*

	2 g/m²[a]	6 g/m²	P
All patients			
ΔLI[b]	1.21[c] (0.29–3.37)[e]	3.24 (0.57–10.4)	<0.00005[d]
No. of patients with ΔLI of			
≥1.2	23/50 (46%)	11/14 (79%)	<0.03[f]
≥1.5	12/50 (24%)	9/14 (64%)	<0.007
≥2.0	5/50 (10%)	7/14 (50%)	<0.0025
CTC patients			
ΔLI	1.37 (0.29–3.37)	3.63 (1.14–10.4)	<0.0007[d]
No. of patients with ΔLI of			
≥1.2	15/26 (58%)	10/12 (83%)	<0.12[f]
≥1.5	9/26 (35%)	9/12 (75%)	<0.025
≥2.0	3/26 (12%)	7/12 (58%)	<0.005

[a] Continuous infusion total dose of ara-C, given with daily daunorubicin, administered over the initial 72 h of therapy.
[b] Change in [³H]dThd LI from pretreatment to day 10 postinitial AcD.
[c] Mean for all patients.
[d] Student's *t* test.
[e] Range for individual patients.
[f] Fisher's exact test.

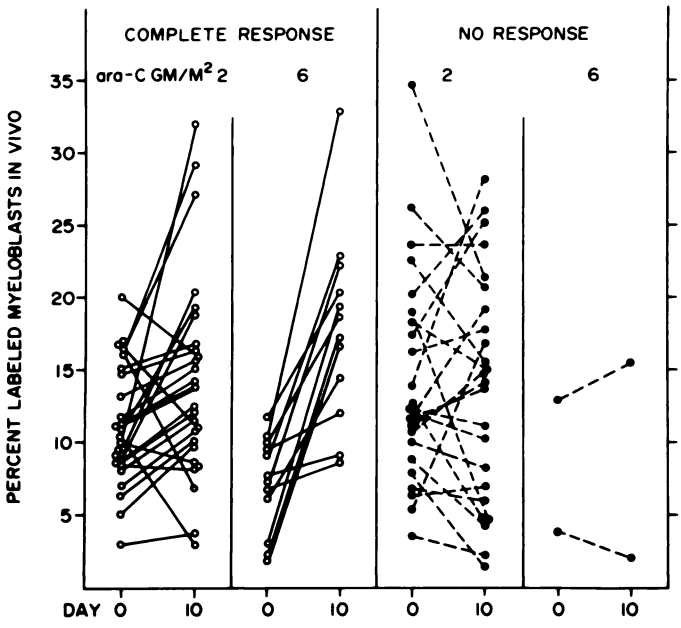

Fig. 1. Pretreatment (day 0) and day 10 post-AcD [H]dThd labeling indices from 50 patients receiving initial 2 g/m²/72 h AcD and 14 patients receiving initial 6 g/m²/72 h AcD. Drug-induced change in LI for each patient is depicted for patients achieving complete response and no response.

DISCUSSION

These clinical laboratory correlates in patients with acute leukemia undergoing timed sequential chemotherapy demonstrate the relationship of increased initial drug dose and greater drug-induced residual tumor proliferation. Our serial *in vivo* and *in vitro* studies in human AML (1–3, 6, 9) and multiple myeloma (7, 11) have demonstrated that these predictable cell kinetic perturbations in DNA synthesis relate both to initial drug-induced cytoreduction and to the concomitant induction of growth-promoting humoral stimulatory activity. The *in vivo* detection of peak drug-induced humoral stimulatory activity coincides with maximal recovering tumor cell [³H]dThd LI and clonogenicity (2, 3, 7, 9, 11). The direct relationship of humoral stimulatory activity to tumor growth has been demonstrated *in vitro* by multiple growth parameters, including [³H]dThd incorporation into DNA and [³H]dThd LI that are ultimately reflected in total tumor cell mass and tumor clonogenic capacity (6, 7, 11). Serial *in vivo* investigations in leukemia-bearing rodents (2–4, 12) have substantiated these related parameters of active tumor growth observed for human hematopoietic malignancies. Our present findings in humans with AML also closely parallel those defined *in vivo* in rodents and similarly correlate the presence and magnitude of drug-primed cell proliferation with complete tumor response.

These findings suggesting that the *in vivo* perturbation of tumor cell proliferation after initial drug is linked with clinical outcome and demonstrating that higher initial drug doses effect

greater drug-induced proliferation further substantiate the applicability of studies in the rat with myelocytic leukemia (2–4). In that model, such dose-dependent increases in residual tumor proliferation conferred enhanced ara-C sensitivity of the maximally stimulated tumor cohort with enhanced disease-free survival from optimally timed sequential drug administration, but not with equal drug given by any other schedule (4, 12).

Our timed sequential approach to therapy of adult AML produces clinical results that are similar to other regimens using ara-C and daunorubicin in combination with respect to the percentage of complete remissions induced (13–16). However, the efficacy of this approach relates not only to overall remission induction rates but to the duration of unmaintained complete remission (9), particularly when a second cycle of timed sequential therapy is given at the time of minimal residual disease in early complete remission (10). The similarity of remission rates achieved by various treatment regimens using ara-C and anthracyclines likely relates to multiple factors, including a significant proportion of myeloid leukemias that are sensitive to the cytotoxic actions of these agents at initial presentation (7, 8), resulting in adequate reduction of measurable tumor burden to achieve complete microscopic tumor clearance and subsequent apparent complete remission. Other pretreatment clinical factors that determine initial outcome during induction therapy (19–22) may also impact on overall survival and confer a homogeneity to response rate. The duration of remission without further drug intervention is a sensitive biological quantification of the volume of tumor kill achieved. The long initial disease-free period (median, ≥30 months) achieved with 2 cycles of AcDAc (10) parallels data from studies in leukemia-bearing rats (12), and suggests that the drug-induced growth perturbations which confer enhanced sensitivity to ara-C are maximally operative at this time of minimal tumor burden.

Our present studies, in combination with previous *in vitro* and *in vivo* rodent and human trials, support the premise that induced proliferation renders residual tumor cells more sensitive to the effects of an optimally timed ara-C infusion. The definition of initial drug dose-related kinetic changes in humans that bear close similarity to *in vivo* correlates in the rodent leukemia model further support our ongoing *in vivo* trials in times sequential therapy in human acute leukemia, where ara-C is administered at the predicted peak of drug-primed residual tumor proliferation (1, 9, 10).

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