Prediction of Therapeutic Response in Acute Myelocytic Leukemia

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Introduction

There have been many attempts to predict the outcome of antileukemic chemotherapy by characterizing the kinetic status or the drug uptake characteristics of a patient's leukemic cells. These attemps have for the most part been unsuccessful [1–4]. In this paper we will present recently acquired data from our laboratories which appears to be useful in predicting the outcome of therapy and will discuss some of the reasons for the failure of previous studies.

Methods

Patients were treated with a combination of adriamycin (ADR) (30 mg/m^2 IV d 1, 2, 3) and cytosine arabinoside (ara-C) (100 mg/m^2/d for 7 days by continuous infusion) [5,6]. Experimental methods are described in the referenced publications.

Results

Pharmacologic Assessment of Therapeutic Sensitivity
Cytosine Arabinoside Phosphorylation and Retention in Vitro

The 4-hour retention of cytosine arabinoside by the leukemic cells of 28 patients was measured (Rustum and Preisler, Cancer Res., submitted 1978). Patients could be divided into two groups: a high retention group-patients whose leukemic cells retained after 4 hrs >30% of their initial levels of ara-CTP, and those whose leukemic cell retention of ara-CTP was low (<20%). Table 1 gives the treatment results for these patients. Both groups were in-

Table 1

Patients	No.	CR	Cause of remission induction failure	Med. dur. CR-Wks.
Entire population	28	20	—	72
High ara-CTP retention (>30%)	13	11	2 pts. died during induction therapy	>78
Low ara-CTP retention (<20%)	15	9	3 pts. with resistant disease (7), 3 pts. died during induction	28

distinguishable with respect to characteristics such as age, initial white blood cell count, etc. It is clear that while the advantage of the high retention group over the low retention group with regards to remission rate was marginal ($p \geq 0{,}05$), the duration of remission for the former patients was much greater than for the latter ($p < 0{,}001$).

Estimation of Ara-CTP Levels Achieved in Vivo with Leukemic Cells

Methods have not as yet been developed which permit direct accurate assessment of the intracellular levels of ara-CTP which are reached during chemotherapy. The intracellular level of ara-CTP achieved during therapy has been determined indirectly by measuring the inhibition of ^{3}H-CdR incorporation into DNA (Rustum et al., unpublished data). An in vitro standard curve is generated for each patient's bone marrow cells prior to initiation of therapy using 0,0625 to 1,0 μg/ml ara-C. The intracellular ara-CTP level achieved in vitro at each concentration is correlated with the extent of inhibition of CdR incorporation into DNA and a standard curve drawn. Twenty-four hours after the initiation of therapy, bone marrow myeloblast cells are obtained from the patient and incubated with CdR-^{3}H to determine the extent of incorporation of CdR into DNA. The rate of incorporation of CdR into this specimen is then related back to the pretherapy in vitro standard curve and an estimation is made of the intracellular level of ara-CTP achieved after 24 hrs of therapy. The results summarized in Table 2 indicate that at an in vitro concentration of 0,0625 μg/ml ara-C the extent of inhibition varied from 8% to 93% in different patients. At 1,0 μg/ml this inhibition varied from 42% to 95%. These results also show that there is a close correlation between the amount of ara-CTP achieved intracellularly in vitro and the extent of inhibition of CdR-^{3}H incorporation into DNA prior to therapy and 24 hrs after the initiation of chemotherapy.

Biological Assessment of Determinants of Response

The development of in vitro clonogenicity assays has permitted the characterization of a population of progenitor cells which have hitherto been impossible to study directly. Since the leukemic CFU-c are a minority progenitor population hidden within the recognizable leukemic cells, studies carried out on the latter population of leukemic cells may not be reflective of the proper-

Table 2

Patient	In vitro percent Inhibition at 0,0625 μg/ml[c]	0,1 μg/ml	In vivo Inhibition[b]	Ara-CTP[a] (pm/10^7 cells)
1	8	45	22	2,8
2	60	83	68	18
3	94	96	93	30

[a] ara-CTP formed intracellularity when bone marrow myeloblasts were incubated with 0,0265 μg/ml ara-C.
[b] 24 hrs. after initiation of therapy.
[c] Ara-C concentration.

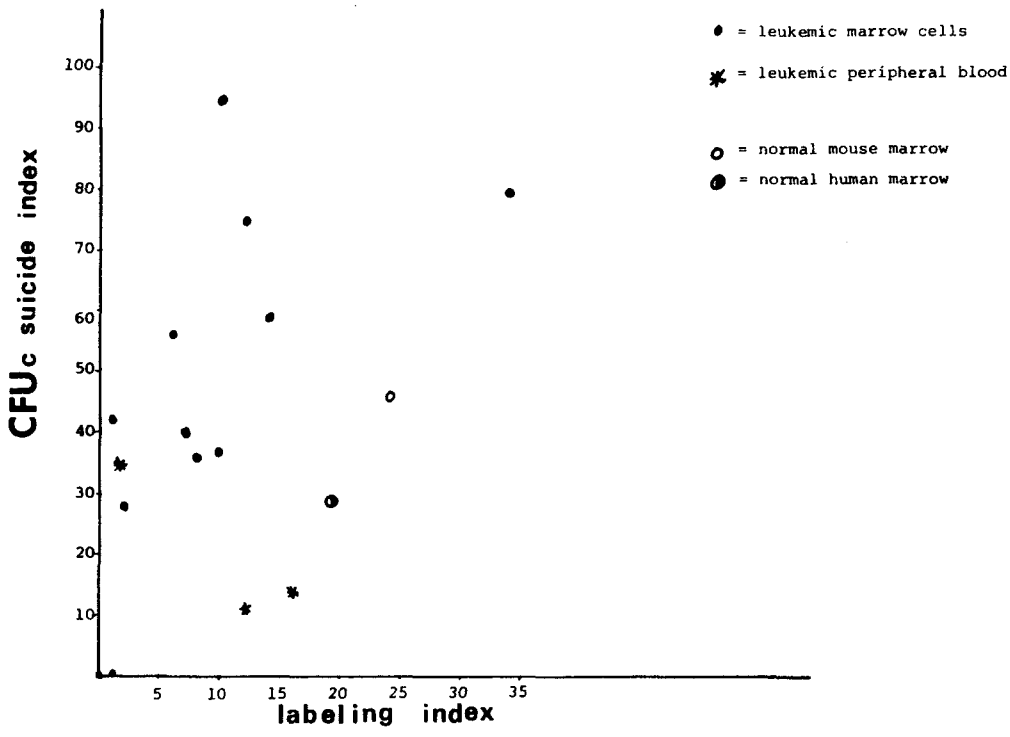

Fig. 1

ties of the leukemic progenitor cells. Support for this proposition has been found in our comparison of the labeling index of the recognizable leukemic marrow cells with the suicide index of the leukemic CFU-c (Preisler and Shoham, submitted to Cancer Res., 1978). Fig. 1 demonstrates that a simple relationship does not exist between the labeling index and the suicide index except in that the labeling index is consistently lower than the suicide index (usually $\frac{1}{2}$–$\frac{1}{7}$).

Estimation of Effects of Adriamycin in Vivo

Hoechst 33342 is a supravital dye which can be used to stain DNA for flow cytometric studies. While comparing leukemic cell DNA histograms before and 24 hrs after the initiation of antileukemia chemotherapy, we found that the degree of DNA fluorescence produced by H33342 was diminished in some patients (Preisler, H.D., Cancer Treat. Rep., in press). In vitro studies were carried out to further characterize this phenomenon. Fig. 2 illustrates one such study. With exposure to increasing concentrations of ADR, there was a progressive decrease in the DNA fluorescence produced by H33342. The effects of exposure to as little as 0,2 μg of adriamycin for 1hr could be detected. As with CdR uptake, we plan to construct a pre-therapy in vitro dose response curve to assess the potential sensitivity of a patient's leukemic cells to ADR. We will also incubate marrow cells with H33342 24 hrs after the initiation of therapy, measure the fluorescence produced and using the pretherapy dose response curve, estimate the in vivo effects of therapy.

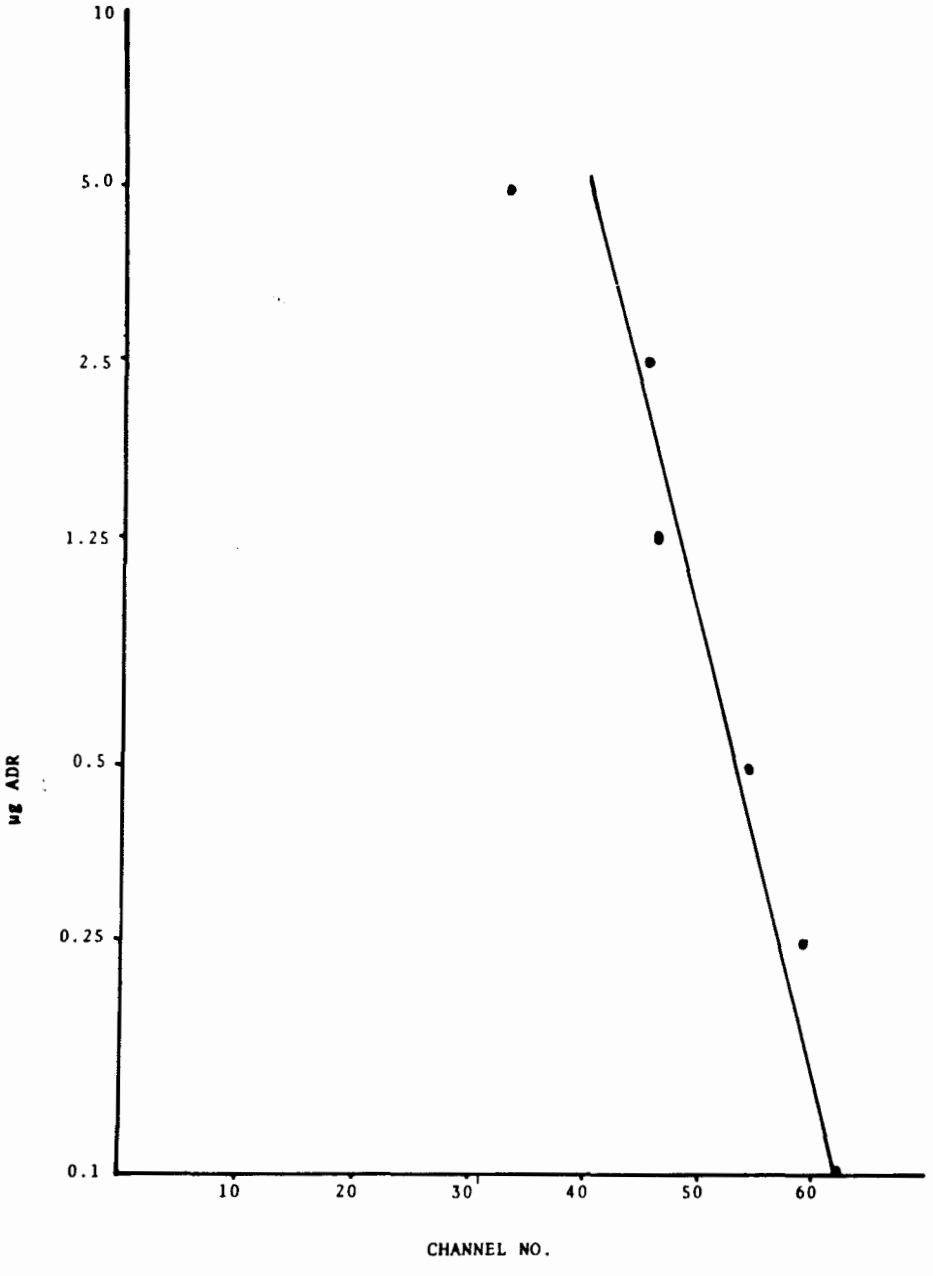

Fig. 2

Discussion

The effectiveness of antileukemia chemotherapy depends upon delivery of the drugs to the target cells, the uptake and activation (if necessary) of the drugs, and the presence of a sensitive metabolic pathway (the latter being dependent in some cases upon the cell cycle characteristics of the leukemic cells). We are attempting to obtain this information by measuring the uptake and activation of ara-C in vitro, deriving an estimate of the in vivo uptake of ara-C and ADR during therapy, and by measuring the labeling index and leukemic CFU-c suicide index prior to and 24 hrs after the initiation of therapy. The latter provides a measure not only of the kinetic status of leukemic CFU-c but also their sensitivity to the antileukemic therapy being employed. Hence, we are attempting to measure the relevant parameters which determine response to therapy.

Two theoretical but practical considerations warrant further discussion. Does measurement of drug uptake by leukemic marrow cells in toto accurately reflect the properties of the leukemic stem cell? This not a moot point since in all likelihood the majority of leukemic cells are end stage cells which are irrelevant with respect to therapeutic attack. The dissociation between the labeling index of the leukemic cells and the suicide index of the clonogenic cells clearly points out that the properties of the easily observable marrow leukemic cells are not necessarily reflective of the properties of the few but crucial stem cells. In our studies to date (and described in this paper), we have found an excellent correlation in previously untreated patients between response to therapy (remission duration – vide infra) and ara-CTP retention by marrow leukemic cells. By contrast, the correlation between ara-C metabolism and response for relapsed patients is not nearly so good and, in fact, in a few cases, high levels of ara-CTP retention have been associated with clinical refractoryness to therapeutic regimens which included ara-C. One possible explanation for this could be a discordance between the drug sensitivity of the leukemic stem cells and the easily observable leukemic cells – a discordance which developed during maintenance therapy. Alternatively, the leukemic cells of these patients may still activate and retain ara-C quite well but may no longer have a sensitive metabolic pathway. Measurement of DNA synthesis (using CdR incorporation) in the presence of ara-C will test this possibility.

The second point requiring discussion relates to evaluation of response to therapy. The end point of all previous studies has been an assessment of whether or not remission has been attained. Considering the aggressive therapeutic regimens currently in use, remission induction rate is an inappropriate end point since marrow aplasia is produced in the vast majority of patients and once this occurs, intercurrent problems relating to infection play a major role in determining whether or not a patient will survive for a sufficiently long period of time to permit regrowth of his bone marrow. Many patients whose leukemic cells are sensitive to therapy die during remission induction [6]. One of us has proposed a system for classifying failure to respond to remission induction therapy so that a distinction can be made with respect to patients who fail remission induction therapy because of leukemic cell resistance to the therapy employed as opposed to those patients who die of intercurrent problems and whose leukemic cells were sensitive to chemotherapy [7]. By contrast the duration of remission appears to be primarily determined by two factors: the number of leukemic cells persisting once remission occurs and the effect of maintenance therapy on residual disease – both of which are reflective of the drug sensitivity of the leukemic cells. Hence, the duration of remission and not the percent remission induction rate is a better measure of leukemic cell sensitivity to drugs. This theoretical construct agrees quite well with our data on ara-CTP retention.

Previous attempts to predict the outcome of antileukemia therapy have not been successful probably because they were based upon the measurement of a single parameter [3,4] (labeling index for example) or used the wrong clinical endpoint [1,2] (remission induction rate). We hope that by

simultaneously determining the relevant cell cycle parameters along with measurements of drug metabolism and the use of appropriate clinical parameters, we will avoid the pitfalls of previous studies and be able to predict the clinical responses of individual patients.

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