Alteration of the Proliferative Rate of Acute Myelogenous Leukemia Cells In Vivo in Patients

By H.D. Preisler, A. Raza, and R.A. Larson

Ten patients with active acute myelogenous leukemia (AML) received either 13-cis retinoic acid (RA) + α interferon (IFN) or recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) for 3 days. Cell cycle measurements were performed before and at the conclusion of administration of the bioactive agent(s). The proliferative rate of the leukemia cells in vivo decreased in four of five patients receiving RA + IFN whereas in one patient proliferation accelerated. The proliferative rate of AML cells accelerated in three of the five patients who received rhGM-CSF and slowed in two patients. These data show that while the proliferative rate of AML cells can be altered in vivo, the effect produced by bioactive agents may be the opposite of the desired effect. Furthermore, the studies described here demonstrate the usefulness of marrow biopsies for measuring the percent S-phase cells and the importance of measuring the duration of S phase so that the effects of bioactive agents on the cell cycle time of the leukemia cells can be determined.

© 1992 by The American Society of Hematology.

TREATMENT OUTCOME in acute myelogenous leukemia (AML) has not improved significantly during the past 10 years. Recently attention has focused on alteration of the proliferative rates of leukemia cells in vivo as a possible means of increasing the effectiveness of chemotherapy. Two opposite but complementary approaches are being evaluated. The most common is to accelerate the proliferative rates of leukemia cells to increase their drug sensitivity.1,2 The second approach is to reduce the regrowth of leukemia cells between courses of cytotoxic chemotherapy by slowing their rate of proliferation.3

This report describes several observations that are critical to investigators who are attempting to alter the proliferative rate of leukemia cells in vivo. These observations are: (1) it is possible to reduce the proliferative rate of leukemia cells; (2) the administration of bioactive agents can produce the opposite effects of that which are intended; and (3) if bioactive agents are to be used effectively, the effects of these agents on the cell cycle time of leukemia cells must be measured. Studies restricted to measuring the percent of cells in S phase are of limited utility.

MATERIALS AND METHODS

Patients had AML according to standard French-American-British (FAB) criteria.3 Patients who received retinoic acid/interferon had experienced more than one leukemic relapse and had not received cytotoxic therapy for at least 1 month before study. Patients who received the recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) were previously untreated patients with poor-prognosis AML. In brief, each of the latter patients was characterized by one or more of the following: age greater than 70 years, leukemia evolving from a preleukemic syndrome (excluding chronic myelogenous leukemia), or leukemia developing subsequent to chemoradiation therapy. Informed consent was obtained from each patient.

Cytokine administration. 13-cis retinoic acid (RA) was administered by mouth at 100 mg/m²/d on days 1, 2, and 3. Interferon (IFN) was administered subcutaneously at 3,000,000 U/m² on days 1 and 3. The RA and IFN were kindly provided by Hoffman-La Roche (Nutley, NJ).

rhGM-CSF was administered intravenously (IV) at 5 μg/kg/d on days 1, 2, and 3 in divided doses subcutaneously every 12 hours. The rhGM-CSF was kindly provided by Dr Richard Huhn of SANDOZ Pharmaceuticals (East Hanover, NJ). One patient (no. 1, Table 1) received neither RA/IFN nor rhGM-CSF. The investigators performing the cell-cycle studies on the leukemia cells of this patient did not know that this patient was a control patient nor did they know which cytokine(s) the other 10 patients received.

Assessment of effects on cell proliferation rates. Before administration of the bioactive agents(s) under study, iododeoxyuridine (IUDR) at 100 mg/m² was infused over 60 minutes. At the end of the infusion a bone marrow (BM) aspirate and a biopsy were obtained. The biopsy was fixed in Bouin's solution and embedded in glycolmethacrylate as previously described.3 The aspirates were incubated with ³HThdr, washed, and placed on coverslips. The cells on one coverslip were reacted with antimodeoxyuridine/IUDR monoclonal antibody 3D9 followed by reaction with a secondary antiamouse antibody conjugated with peroxidase. A second coverslip was treated in an identical manner followed by dipping in photographic emulsion and by autoradiography. The number of singly and doubly labeled cells were assessed as previously described, and the percent cells in S phase (LI) and the duration of S phase (Ts) were measured and the overall cell-cycle time (Tc) calculated.1

After the 3-day administration of the bioactive agent(s), bromodeoxyuridine (BrdU) 100 mg/m² was infused IV over 1 hour. At the end of the infusion a BM aspirate and biopsy were obtained and processed as described above with the exception that the primary antibody (Br-3) used was reactive only with BrdU. Because this antibody reacts only with BrdU, at day 3 one can calculate the Ts and Tc without interference from IUDR, which may still be present in the leukemia cells.

RESULTS

Effects of bioactive agents on leukemia cell proliferation rates. Tables 1 and 2 illustrate the effects of RA/IFN and rhGM-CSF on AML cells in vivo. Patient no. 1 (Table 1) was a control patient receiving neither RA/IFN nor rhGM-CSF. The cell-cycle characteristics of this patient's leukemia cells, as assessed by using BM biopsy data, were...
indistinguishable at \( t = 0 \) and \( t = 3 \) days. In contrast, if the BM aspirate LI had been used, the cell-cycle characteristics would appear to have changed between \( t = 0 \) and \( t = 3 \) days. For this reason, among others, the following description of the effects of bioactive agents on proliferative characteristics refer to measurements using the BM biopsy LI. The section that follows provides a more complete description of the problems associated with the use of BM aspirates or peripheral blood cells to measure the cell-cycle effects of bioactive agents.

With respect to RA/IFN, the administration of these agents was associated with a slowing of the proliferative rate of the leukemia cells in four of five patients and with proliferation acceleration in one patient (Table 1). In three of these patients, the proliferative rate was slowed to less than one half the baseline rate. No consistent relationship between the effect of RA/IFN on cell cycle characteristics and on the white blood cell count was detected. Considering the four cases for whom data are available, the administration of RA/IFN was associated with a substantial increase in the absolute number of circulating blast cells in two cases and perhaps slight increases in two cases. An increase in the absolute number of mature myeloid cells in the blood was detected in three of four patients; however, the increase was small, being less than twofold in every case and was less than 2,000/\( \mu \)L in each.

The administration of rhGM-CSF increased the proliferative rate of the leukemia cells in three patients, doubling the rate in patient nos. 9 and 11 (Table 2). In two patients (nos. 7 and 8) the proliferative rate (Tc) slowed, dramati-
ally so in patient no. 8 (from 39 hours to 119 hours). The percentage of mature granulocytes in the peripheral blood increased in four of five patients whereas the absolute number increased in all five, more than doubling in three. The absolute number increased by greater than 2,000/μL in three patients. In contrast, the proportion of blast cells in the peripheral blood increased in only two of five patients whereas the number of circulating blast cells increased in three. Of interest is the fact that the number of blast cells in the blood increased in only those three patients in whom rhGM-CSF administration was associated with proliferation acceleration (nos. 9 through 11).

Measurement of the effects of bioactive agents in vivo. The LI of the aspirate cells of the control patient (no. 1 in Table 1) increased from 2% at t = 0 to 4.7% at t = 3 days. If these data are used to calculate the cell-cycle time, then the t = 0 cell-cycle time is extraordinarily long (485 hours) and appears to have decreased (accelerated) to 196 hours during the 3-day control period. Use of the aspirate LI also results in the calculation of extraordinarily long initial cell-cycle times for patient no. 2 (Table 1, 596 hours) and in patient no. 8 (Table 2, 383 hours).

Considering all patients, the correlation between the LI of the marrow biopsy and that of the marrow aspirate is low (r = .4836, P = .01). When the effects of RA/IFN and rhGM-CSF on the cell-cycle time and on the LI of the marrow biopsy are considered, the changes were concordant in 9 of the 10 studies, with the LI decreasing when the proliferative rate slowed and increasing when proliferation accelerated. In contrast, the changes in the LI of the marrow aspirate and in cell-cycle times were discordant in three of eight studies (patient nos. 3, 8, and 10). The effects of bioactive agent(s) on the LI of the peripheral blood cells were discordant from those on the LI of the BM biopsy in three of eight comparisons and discordant in three of eight comparisons with BM aspirates.

While the directional changes in the marrow biopsy LI and in the cell-cycle time were usually concordant, the degree of change was not proportional. For example, in patient nos. 5 and 6 (Table 1) the LI decreased by 60%, while the cell-cycle time slowed fourfold to fivefold. A similar quantitative discordance was noted for patient no. 8.

DISCUSSION

The data presented here show that it is possible to significantly slow or accelerate the proliferative rate of leukemia cells in patients. The extent of slowing is compatible with that projected to be necessary to produce a net increase in the efficacy of cytotoxic therapy.6

While it has been tacitly assumed that bioactive agents would either produce the desired effect on leukemia cells or produce no effect at all, this has proved not to be the case because in two patients administration of rhGM-CSF resulted in a slowing of the proliferative rate of the leukemia cells and in one patient the administration of RA/IFN accelerated leukemia cell proliferation. A discordance was noted between in vivo and in vitro effects of rhGM-CSF in that rhGM-CSF administration to patient no. 8 produced a dramatic slowing of the proliferative rate of the leukemia cells, while the leukemia cells failed to proliferate in vitro in the absence of rhGM-CSF but produced 220 colonies/2 × 10^5 cells in its presence. While the proliferation inhibition effects of rhGM-CSF were not expected, the proliferation stimulatory effects of RA/IFN are compatible with the reports that IFN administration can increase the white blood cell count in some AML patients7 and that RA may stimulate leukemia cell proliferation in vitro.8

The ability of bioactive agents to produce in vivo effects that are the opposite of those which are desired greatly complicates the analysis of studies designed to evaluate the clinical efficacy of these agents. For example, if rhGM-CSF increases the chemotherapeutic sensitivity of the leukemia cells of some patients by accelerating proliferation, then a decrease in the proliferative rate should reduce drug sensitivity. In a clinical trial these effects will offset each other, possibly resulting in the erroneous conclusion that rhGM-CSF is clinically ineffective even though it may produce highly beneficial effects in some patients. To avoid erroneous conclusions, one must have the capacity to identify those patients in whom the bioactive agent produces the desired effect.

The data presented here, together with previously published studies, strongly suggest that BM aspirates should not be used to evaluate the effects of bioactive agents on leukemia cells in vivo. The spuriously low percent of S-phase cells in the aspirate results in the calculation of inordinately long cell-cycle times. Additionally, because the degree of dilution of the marrow cells by peripheral blood cells varies from aspirate to aspirate,9,10 sequential aspirates cannot be used to reliably measure the effects of bioactive agents on the proportion of cells in S phase. This is clearly illustrated by the fact that if aspirate data were used, completely spurious data would have been derived from the studies of 4 of the 11 patients studied here (nos. 1, 3, 7, and 8). Similar problems are associated with the use of peripheral blood cells for these studies. An additional problem with most contemporary studies of bioactive agents is that only the percent of cells in S phase is measured while the duration of S phase and of the cell cycle is not.12,18 Because the most important effects of these agents are on the cell-cycle time and because (as in patient no. 7) the effects on the cell-cycle time and on the percent cells in S phase may be the opposite, it is essential that measurements not be restricted to the labeling index.

REFERENCES

ALTERATION OF LEUKEMIA CELL PROLIFERATION


