In Vitro Drug Sensitivity Studies of Colony-forming Units in Culture in Chronic Myelocytic Leukemia: Lack of Specificity between Chronic-Phase Patients and Normal Donors

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ABSTRACT

Attempts to eliminate Philadelphia chromosome-positive cells during the treatment of chronic-phase chronic myelocytic leukemia (CML) have been largely unsuccessful, probably due to the lack of specificity of drugs which have been used. In an attempt to develop more specific therapy for CML, an assay for colony-forming units in culture was used to test for differences between CML blood and normal marrow progenitor cells. The following drugs, which have activity in acute nonlymphocytic leukemia, were tested over a range of concentrations achievable in vivo: Adriamycin; 1-O-α-arabinofuranosylcytosine; aclacinomycin; m-(4-acridinylamino)-3-methoxyphenyl methansulfonamide; methylglyoxalbis(guanylhydrazone), and 5-azacytidine. [3H]Thymidine suicide indices were also determined. Normal marrow colony-forming units in culture tended to be more sensitive to all the drugs which were tested, although not of statistical significance. There was no difference in the suicide index between CML and normal colony-forming units in culture. It is concluded that the drugs which were tested are not likely to selectively kill CML progenitor cells while permitting normal hematopoietic elements to survive.

INTRODUCTION

The prognosis of patients with CML has changed very little since the introduction of busulfan over 30 years ago (7). Attempts to eliminate Philadelphia chromosome-positive cells during chronic phase with aggressive chemotherapy have been largely unsuccessful (1). It is probable that the drugs which have been used were nonspecific for killing CML cells as opposed to residual normal hematopoietic cells. In an attempt to develop more specific therapy for CML, we have used a CFUc assay to test for differences in drug sensitivity in vitro between CML blood and normal marrow myeloid progenitor cells.

MATERIALS AND METHODS

Samples. Peripheral blood was obtained from 18 CML patients, all of whom were Philadelphia chromosome positive, during chronic phase, when their WBC exceeded 50,000/µl. Five healthy males donated bone marrow for control studies. Informed consent was obtained from the patients and donors.

Cell Preparation. As described previously (8, 9), light density mononuclear cells were obtained by centrifuging citrate-anticoagulated blood or marrow through Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, N. J.) (specific gravity, 1.077 g/ml). The interface cells were washed twice and suspended in Roswell Park Memorial Institute Tissue Culture Medium 1640 with 10% fetal calf serum at a concentration of 5 x 10^6/ml. After a 1-hr incubation at 37°C in a humidified atmosphere of 5% CO₂-95% air, appropriate amounts of the drugs were added to make the final concentrations as planned. The drugs were obtained commercially (1-α-arabinofuranosylcytosine, The Upjohn Co., Kalamazoo, Mich.; Adriamycin, Adria Laboratories, Inc., Wilmington, Del.) or from the National Cancer Institute [m-(4-acridinylamino)-3-methoxyphenyl methansulfamide and methylglyoxalbis(guanylhydrazone)] and Bristol Laboratories, Syracuse, N. Y. (5-azacytidine and aclacinomycin). The drugs were dissolved in sterile water or 0.9% NaCl solution and brought to final concentration with Eagle's MEM. For determinations of SIs, [3H]thymidine (55 Ci/mmol; 0.5 mCi/ml) was added to the suspended cells. To a control tube of cells which served as a control, 0.9% NaCl solution was added. After a 1-hr incubation with the drugs or 0.9% NaCl solution, the cells were pelleted, washed with medium, and then resuspended at a concentration of 10^6 cells/ml for cloning studies.

Cloning in Agar. A total of 10^5 cells was added to 0.85 ml of Eagle's MEM with 15% fetal calf serum and 0.3% agar and plated in triplicate over a 2.5-ml overlayer of Eagle's MEM with 15% fetal calf serum and 20% giant cell tumor-conditioned medium as a source of colony-stimulating factor (2). The cultures were incubated in a humidified atmosphere of 5% CO₂-95% air at 37°C for 7 days and then fixed with 3% gluteraldehyde. The plates were coded and then counted by a single individual using an inverted microscope. The number of clusters (4 to 20 cells) was determined for each drug. This increased the number of clones in the control cultures, which exceeded 50 in each case.

The Student t test was used for statistical analysis.

RESULTS

The killing of CML and normal CFUc (expressed as percentage) by the various drugs is presented in Table 1. There was no difference in the SI between the CML and normal CFUc nor were there any statistically significant differences in the percentage of killing by any of the drugs at any concentration. For each drug, however, there was a tendency for the normal marrow CFUc to be more sensitive than the CML CFUc.

For 1-α-arabinofuranosylcytosine, there was a correlation between the SI and the percentage of killing at 0.3 and 3.0 µg/ml (r = 0.8; p < 0.05). There was also a correlation between the SI and the percentage of killing by adriamycin at 0.3 µg/ml (r = 0.9; p < 0.001). For the 1-α-arabinofuranosylcytosine-Adriamycin combination and for the other drugs, there was no correlation between the SI and the percentage of killing.
the CFU-GEMM (3). Unfortunately, the cloning efficiency of these drugs on the cell which gives rise to mixed colonies in vitro, tent stem cell (4), it may be more appropriate to study the effects of drugs on the CML patient, chronic myelogenous leukemia (CML) clone could be more sensitive than a residual normal clone. It is thus unlikely that they will be of value in ablating normal CFUc to be more, rather than less, sensitive to these agents than normal marrow CFUc. If normal hematopoietic progenitor cells are present in patients with CML and if a drug killed selectively the neoplastic clone of cells while relatively sparing the normal clone, then the drug could be administered during chronic phase in an attempt to reduce the CML clone and prolong the chronic phase of the illness. These in vitro studies provide data indicating that normal marrow myeloid progenitors appear to be more, rather than less, sensitive to these agents than the CML CFUc. It is thus unlikely that they will be of value in ablating the neoplastic clone while permitting normal hematopoietic progenitor cells to survive.

This conclusion must be interpreted with caution, however, for several reasons. There was variation in drug sensitivity among the CML patients, and the entire set of patients was compared to a group of normal donors (where there was less variability in drug sensitivity). It may be that, for any individual patient, the CML clone could be more sensitive than a residual normal clone. Unfortunately, there is currently no method to clone sufficient numbers of Philadelphia chromosome-negative cells from most CML patients. Moreover, there is evidence that, in some cases, the Philadelphia chromosome-negative cells may in fact be derived from a neoplastic clone (5). An additional consideration in interpreting the present data concerns the CFUc as the target cell for in vitro drug sensitivity. Since it has been well demonstrated that the neoplastic process in CML involves the pluripotent stem cell (4), it may be more appropriate to study the effects of drugs on the cell which gives rise to mixed colonies in vitro, the CFU-GEMM (3). Unfortunately, the cloning efficiency of these progenitor cells is insufficient to perform statistically analyzable studies at present (10).

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