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Radiosensitivity of Drug-resistant L1210 Leukemia

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Summary

The radiation response of murine L1210 leukemia was examined. Radiation dose-survival curves demonstrated that nondividing (resting) leukemic cells and logarithmically proliferating leukemic cells were equally radiosensitive. A radiosensitive state was also demonstrated for 2 drug-altered leukemia cell lines, 1 strain being markedly resistant to antimetabolite therapy and the other having a decreased growth rate following exposure to an alkylating agent. These studies indicate that cross-resistance does not exist in this experimental system under conditions where drug resistance is present.

Introduction

The therapy of human acute leukemia has resulted in a significant prolongation of survival. One of the encouraging achievements is the markedly increased duration of unmaintained remissions attained using combinations of chemotherapeutic agents (6). This suggests that a significant reduction in the leukemic cell populations was achieved, and there is experimental evidence that perhaps complete eradication of the cellular phase of the disease was being approached (12). Likewise, maintenance chemotherapy following remission induction has become more effective with increasing attention to dose scheduling (19). Nonetheless, it is discouraging that, with few exceptions, relapse of disease occurs following treatment. Also, autopsy studies of patients dying from secondary causes while in clinical remission of disease (normal bone marrow) have demonstrated the presence of residual leukemic infiltrates in various organ systems (16).

It is of interest to consider the theoretic possibilities explaining the failure of chemotherapy to achieve eradication of all leukemic cells. It has been shown experimentally (18) that nondividing leukemic cells *in vitro* are relatively resistant to antimetabolite therapy. This is expected since the effect of antimetabolites is dependent upon nucleic acid synthesis. It may be surmised that leukemic cells *in vivo* in a nondividing or "resting" phase or cells sublethally damaged by chemotherapy and having a decreased proliferative rate (11, 13) would also be refractory to chemotherapy. A 2nd mode of resistance relates to enzyme levels as exemplified by an increased enzyme concentration in amethopter in resistance (15) and a deleted enzyme in 6-mercaptopurine resistance (4). A 3rd potential mechanism causing therapeutic failure is the inability to achieve adequate drug levels in certain sites. The problem of meningeal leukemia created by the blood-brain barrier is well recognized (20, 21). A similar situation may well exist with respect to tissue or lymph node aggregates of leukemic cells (3). The question of reinduction of disease by an inciting agent is perhaps academic at present since chemotherapy has not proven capable of systematically eradicating the cellular phase of leukemia in humans. Until this can be accomplished, the importance of reinduction of disease cannot be critically appraised.

In considering these explanations for therapeutic failure (and others may well exist), it seems appropriate that approaches other than further increases in drug dosages or repeated modifications of drug schedules should be considered. For example, ionizing radiation has not been critically evaluated in human acute leukemia. In a few limited clinical studies, patients have been irradiated with massive single exposures. These patients, treated in the terminal phase of their disease, received minimal benefit, although objective responses were observed (1, 9). However, the potential value of radiation (as of certain chemotherapeutic agents) in acute leukemia may be in selected phases of treatment. It may be that, rather than being clinically useful for remission induction, total body irradiation could be better exploited in remission maintenance or as part of an intensive treatment program designed to hopefully eradicate the cellular phase of the disease.

The present investigation was intended to experimentally evaluate the radiation response of a murine leukemia under conditions where drug resistance is present. To determine if cross-resistance existed, the radiosensitivity of both dividing (*in vivo*) and nondividing (*in vitro*) leukemic cells was examined. In addition, 2 drug-altered L1210 cell lines, 1 of which is markedly resistant to antimetabolite chemotherapy, were studied.

Materials and Methods

Male DBA/2 mice, weighing 20–25 gm and approximately 10 weeks old, were used in all experiments. They were housed in plastic cages in a constant temperature facility and were provided with water and laboratory chow *ad libitum*. The leukemia L1210 strain used is carried as an ascites line with weekly transfer i.p. Experimental mice dying were examined for gross evidence of leukemia (bloody ascites, splenomegaly, lymphadenopathy). Irradiations were performed with 2.5-mv X-rays from a Van de Graaff generator (HVL = 9.6 mm Pb). Dosimetry was performed with the ferrous sulfate chemical dosimeter, and a dose rate of 280 rads/min was used.

Radiation dose-survival curves were performed using the technic developed by Hewitt and Wilson (7) and applied by others (2) to ascites leukemia tumors. Essentially, the technic consisted of irradiating leukemia cells either *in vivo* or *in vitro*. A cell count was then performed on a tumor cell suspension prepared in ice-cold normal saline. Serial dilutions were made to bracket the inoculum concentrations where 50% of the recipient animals

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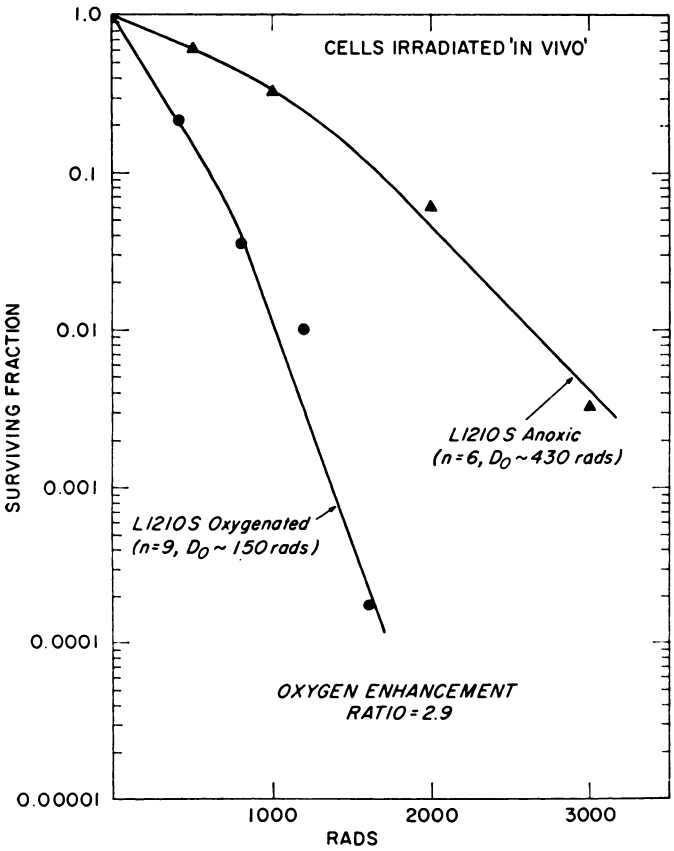

CHART 1. Irradiation of the drug-sensitive strain (L1210S) *in vivo* under oxygenated and anoxic conditions. The dependence of the radiation response on the presence of oxygen is clearly evident with an oxygen enhancement ratio of 2.9 (D_O anoxic/D_O oxygenated). (D_O, the dose reducing the surviving fraction 63% along the exponential portion of the curve; n, the extrapolation number.)

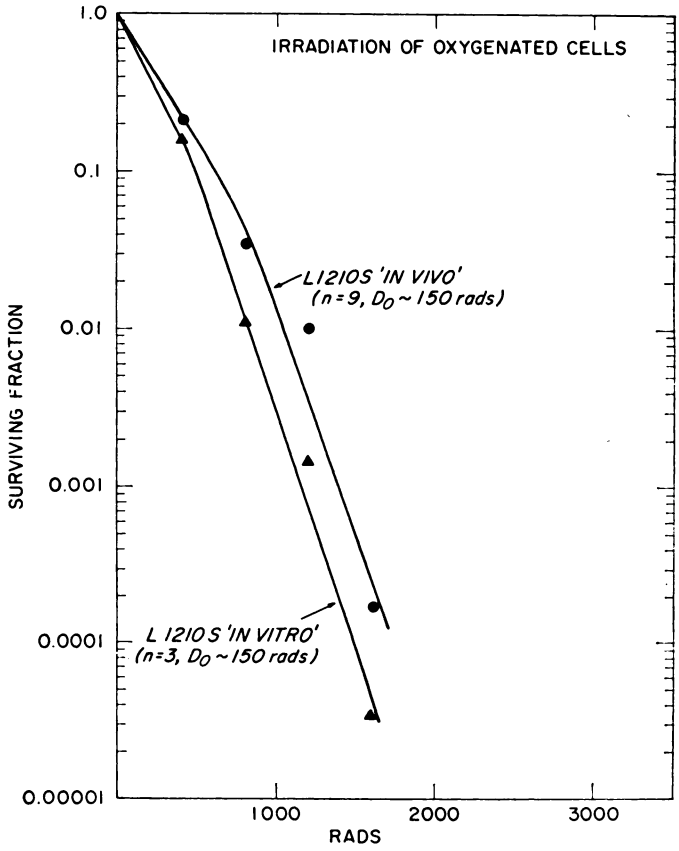

CHART 2. Irradiation of the drug-sensitive strain (L1210S) under conditions of logarithmic cell division (*in vivo*) and nondivision (*in vivo*). The leukemic cells are oxygenated under both conditions and have approximately equal radiosensitivity. (D_O, the dose reducing the surviving fraction 63% along the exponential portion of the curve; n, the extrapolation number.)

developed leukemia at each radiation (and control) dosage. From 9 to 11 normal mice were inoculated per individual dilution, and these assay mice were observed for 80 days for the development of leukemia (a single unirradiated leukemic cell produces death in approximately 15 days). The TD_{50} (tumor dose 50%), the number of cells required to transmit leukemia to one-half the recipient mice, was established by the Reed-Muench method (17). The ratio of the control TD_{50} (unirradiated cells) to the TD_{50} after varying radiation dosages provided the surviving fractions which defined the survival curves. A control TD_{50} was determined for each independent experiment, and the approximate characteristic of each survival curve has been confirmed by at least 1 independent experiment. The survival curves are described by the 2 conventional parameters, the dose reducing the surviving fraction 63% along the exponential portion of the curve (D_0) and the extrapolation number (n).

Results

Survival curves were initially investigated for L1210 leukemia by irradiating leukemic cells *in vivo* using early (3 days postinoculation of 10^5 cells i.p.) and advanced (7 days postinoculation of 10^5 cells i.p.) tumors. It has been reported (2) for another experimental leukemia that the cells of a tumor with appreciable ascites formation are markedly hypoxic. The known dependence of radiation response on tissue oxygenation (5, 8) is depicted for leukemia L1210 in Chart 1. The oxygenated leukemic cells in the early tumor show a markedly greater radiosensitivity than for the advanced tumor with an oxygen enhancement ratio of 2.9.

Growth curve determinations using the technic of Klein and Revesz (14) have shown a 3-day tumor to be in logarithmic growth phase. The survival curves for irradiation of such logarithmically proliferating cells *in vivo* and of nondividing cells *in vitro* are shown in Chart 2. The leukemic cells are well oxygenated in both situations, and the similar survival curves demonstrate that the radiosensitivity of a nonsynchronous leukemia cell population does not significantly depend on a state of active cell division. The *in vitro* survival curve for a slowly dividing strain isolated following exposure to cyclophosphamide suggests a degree of radiosensitivity which is at least comparable to that of the parent L1210 strain (Chart 3). The doubling time of cell line CTX 257 is 24 hr, approximately twice that of the untreated strain.

An L1210 substrain (C95K), markedly resistant to amethopterin and 6-mercaptopurine and partially resistant to cyclophosphamide, was similarly studied. Growth curves demonstrated

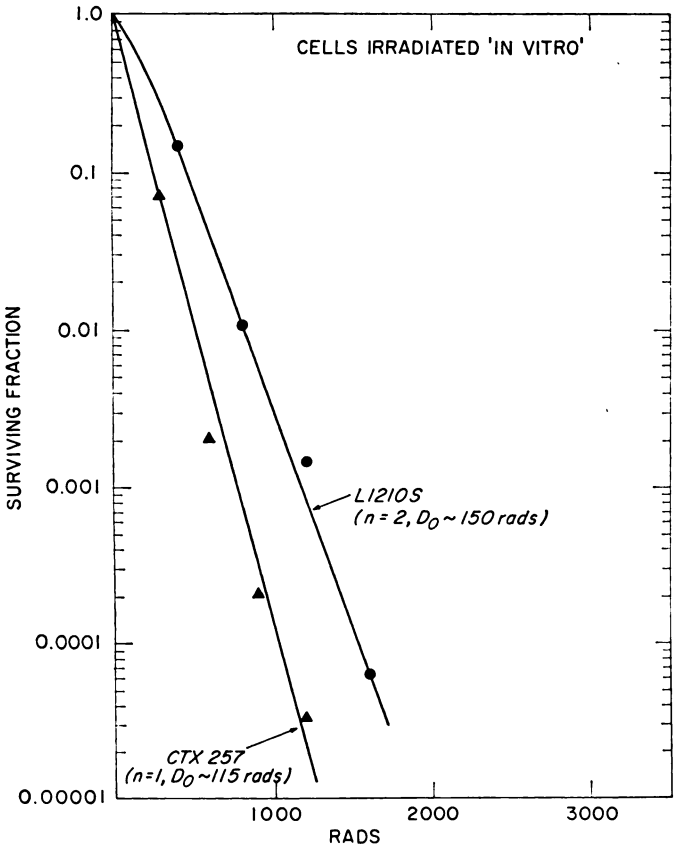

CHART 3. *In vitro* irradiation of the drug-sensitive strain (L1210S) and Substrain CTX 257 isolated following cyclophosphamide exposure and having a decreased growth rate. The latter cell line is at least as radiosensitive as the parent strain. (D_0, the dose reducing the surviving fraction 63% along the exponential portion of the curve; n, the extrapolation number.)

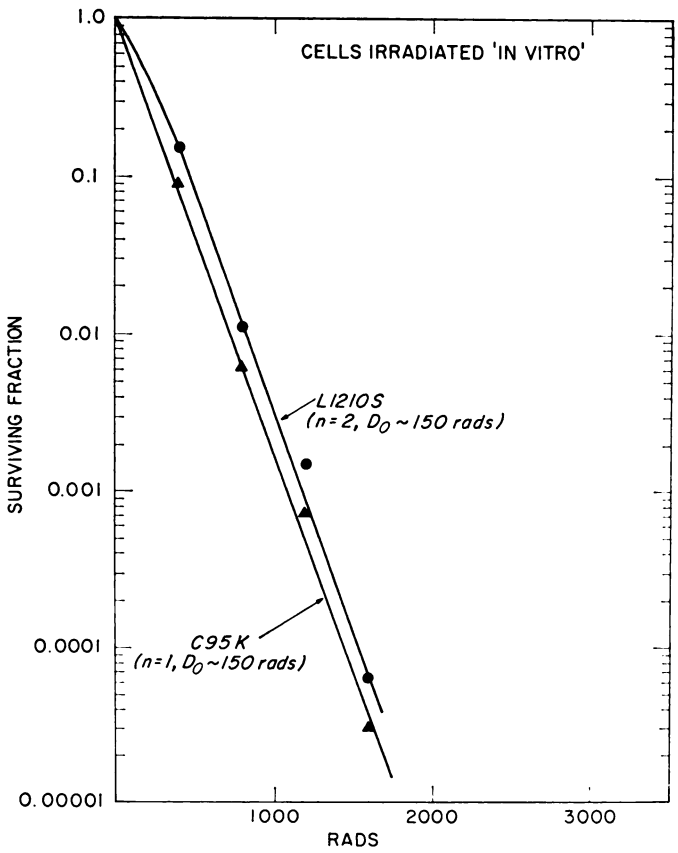

CHART 4. *In vitro* irradiation of the drug-sensitive strain (L1210S) and a drug-resistant substrain (C95K). The similar survival curves indicate the absence of cross-resistance to radiation. (D_0, the dose reducing the surviving fraction 63% along the exponential portion of the curve; n, the extrapolation number.)

TABLE 1

AMETHOPTERIN THERAPY OF EARLY L1210 LEUKEMIA[a]

Tumor line[b]	Dose	No. of mice	Median survival time	70 day survivors
L1210S	Control	50	14.5	1/50
	125 mg/kg	100	21.0	33/100
C95K	Control	50	14.5	3/50
	125 mg/kg	100	15.5	4/100

[a] 10^3 cells s.c. with drug given i.p. on Day 3.

[b] L1210 is a drug-sensitive cell line, and C95K, a drug-resistant mutant line.

that both the sensitive strain (L1210S) and the drug-resistant substrain have the same growth rate (doubling times of approximately 12 hr). The degree of amethopterin resistance of the C95K cell line is indicated in Table 1. A significantly reduced number of curves and minimal increase in the median survival time were obtained with the drug-resistant cell line, using the same tumor inoculum size and day of treatment and the identical drug preparation. Chart 4 shows that the radiation survival curve for this triple drug-resistant tumor is similar to the drug-sensitive strain under *in vitro* conditions. The survival curves were also comparable for the C95K and L1210S cell lines under oxygenated *in vivo* conditions.

Discussion

These studies demonstrate that there is no qualitative difference in the radiosensitivity of L1210 leukemia under certain conditions where refractoriness to chemotherapy exists. Nondividing leukemic cells have the same radiation survival curve as cells irradiated during logarithmic growth. This relative independence of radiation effect on the proliferative state contrasts with the dependence of antimetabolite effect on an actively dividing tumor cell population. Likewise, no radioresistance was apparent for the triple drug-resistant substrain. It is of interest that the shoulder of the survival curves for the drug-altered cell lines (CTX 257 and C95K) appeared to be minimal or absent. The explanation for this finding, which was observed on repeated experiments, will require further investigation and confirmation in other drug-exposed cell lines.

The absence of cross-resistance to radiation under the above conditions is an experimental indication that total body irradiation may be advantageously combined with chemotherapy in the treatment of human acute leukemia. It has also been shown

experimentally (10) that radiation can eradicate leukemic cells in the central nervous system where inadequate drug concentrations are attained with systemic chemotherapy. It is therefore suggested that additional laboratory studies be undertaken to investigate the experimental basis for radiotherapy as a potential adjunct in the clinical treatment of leukemia.

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References

1. Andrews, G. A., Sitterson, B. W., White, D. A., Kniseley, R. M., and Comas, F. V. Summary of Clinical Total-Body Irradiation Program, Oak Ridge Institute of Nuclear Studies, Medical Division, Research Report for 1962.
2. Berry, R. J., and Andrews, J. R. Quantitative Relationships Between Radiation Dose and Reproductive Capacity of Tumor Cells in a Mammalian System *In Vivo*. Radiology, *77:* 824–30, 1961.
3. Block, J. H., Miller, J. K., Harris, A. R., Berlin, N. I., and White, J. Water Exchange in Animal and Human Tumors. J. Appl. Physiol., *16:* 181–85, 1965.
4. Brockman, R. W. Biochemical Aspects of Mercaptopurine Inhibition and Resistance. Cancer Res., *23:* 1911–1201, 1963.
5. Gray. L, H., Conger, A. D., Ebert, M., Hornsby, S., and Scott, 0. A. The Concentration of Oxygen Dissolved in Tissues at the Time of Irradiation as a Factor in Radiotherapy. Brit. J. Radiol., *21:* 638–48, 1953.
6. Henderson, E. S., Freireich, E. J., Karon, M., and Rosse, W. High Dose Combination Chemotherapy in Acute Lymphocytic Leukemia of Children. Proc. Am. Assoc. Cancer Res., *7:* 30, 1966
7. Hewitt, H. B., and Wilson, C. W. A Survival Curve for Mammalian Leukemia Cells Irradiated '*In Vivo*' (Implications for the Treatment of Mouse Leukemia by Whole-Body Irradiation). Brit. J. Cancer, *13:* 69–75, 1959.
8. Hollcroft, J. W., Lorenz, E., and Mathews, M. Factors Modifying the Effect of X-Irradiation on Regression of a Transplanted Lymphosarcoma. J. Natl. Cancer Inst., *12:* 751–763, 1952.
9. Jacobs, M. L., and Marasso, F. J. Four Year Experience with Total Body Irradiation. Radiology, *84:* 452–456, 1965.
10. Johnson, R. E. An Experimental Therapeutic Approach to L1210 Leukemia in Mice: Combined Chemotherapy and Central Nervous System Irradiation. J. Natl. Cancer Inst., *32:* 1333–41, 1964.
11. Johnson, R. E., Hardy, W. G. and Zelen, M. Chemotherapeutic Effects of Mammalian Tumor Cells. III. Modification of Leukemia L1210 Growth Kinetics with an Antimetabolite. *Ibid.*, *36:* 15–20, 1966.
12. Johnson, R. E., Zelen, M., and Freireich, E. J. An Evaluation of Human Acute Leukemia Data Using a Murine Leukemia Model System. Cancer, *19:* 481–84, 1966.
13. Johnson, R. E., Zelen, M., and Kemp, N. H. Chemotherapeutic Effects of Mammalian Tumor Cells. I. Modification of Leukemia L1210 Growth Kinetics and Karyotype with an Alkylating Agent. J. Natl. Cancer Inst., *34:* 277–90, 1965.
14. Klein, G., and Revesz, L. Quantitative Studies on the Multiplication of Neoplastic Cells *In Vivo*. I. Growth Curves of the Ehrlich and MCIM Ascites Tumors. *Ibid.*, *14:* 229–73, 1953.
15. Misra, D. K., Humphreys, S. R., Freidkin, M., Goldin, A., and Crawford, E. J. Increased Dihydrofolate Reductase Activity as a Possible Basis of Drug Resistance in Leukemia. Nature, *189:* 39–42, 1961.
16. Nies, B. A., Bodey, G. P., Thomas, L. B., Brecher, G., and Freireich, E. J. The Persistence of Extramedullary Leukemic Infiltrates During Bone Marrow Remission of Acute Leukemia. Blood, *26:* 133–41, 1965.
17. Reed, L. J., and Muench, H. A Simple Method of Estimating Fifty Per Cent Endpoints. Am. J. Hyg., *27:* 493–97, 1938.
18. Schabel, F. M., Skipper, H. E., Trader, M. W., and Wilcox, W. S. Experimental Evaluation of Potential Anticancer Agents. XIX. Sensitivity of Nondividing Leukemic Cell Populations to Certain Classes of Drugs *In Vivo*. Cancer Chemotherapy Rept., *48:* 17–30, 1965.
19. Selawry, O. S., and Frei, E. Prolongation of Remission in Acute Lymphocytic Leukemia by Alteration in Dose Schedule and Route of Administration of Methotrexate. Clin. Res., *12:* 231, 1964.
20. Thomas, L. B., Chirigos, M. A., Hymphreys, S. R., and Goldin, A. Pathology of the Spread of L1210 Leukemia in the Central Nervous System of Mice and Effects of Treatment with Cytoxan. J. Natl. Cancer Inst., *28:* 1355–89, 1962.
21. Whiteside, J. A., Phillips, F. S., Dargeon, H. W., and Burchenal, J. H. Intrathecal Amethopter in Neurological Manifestations of Leukemia. Arch. Internal Med., *101:* 279–85, 1958.

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