Predictive Values of the in Vivo Diffusion Chamber for Cyclophosphamide Treatment of L1210 Murine Leukemia

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ABSTRACT

In vivo culture of tumor cells using the Millipore diffusion chamber implanted i.p. into female C57BL x DBA/2 F1 (hereafter called BD2F1) mice provides a means for direct examination of drug effect on tumor cells. The effect of various doses and schedules of i.p. cyclophosphamide (CY) on murine L1210 leukemia cell count in the chambers was compared to survival of leukemia-bearing animals treated similarly. Tumor cell viability was assessed by transfer of chamber contents to recipient animals who were then observed for survival. Tumor cell viability was assessed by direct counting of cells remaining in chambers. Single doses proved to be more effective than were equally divided doses in decreasing chamber cell number and prolonging leukemic animal survival. Rejection of L1210 cells rescued from chambers after host treatment with CY revealed that many could not produce tumors. Results suggest that this technique provides reproducible information on drug effects and may be a valuable tool for designing clinically useful dose schedules.

INTRODUCTION

The effect of chemotherapeutic agents on animal tumors is not always predictable of their clinical usefulness for analogous human tumors. Drug effect on either human or analogous animal tumor cells cultured in vitro also may not accurately reflect in vivo usefulness. A diffusion chamber system (1, 2, 12, 16) may provide such a method for evaluation of in vivo drug effect on human cells. Normal human bone marrow and hematopoietic cells (5, 15, 26), as well as human leukemic (9, 17, 18, 22, 27, 28), HeLa (24), melanoma (29), myeloma (19), choriocarcinoma (21, 22), gastric (20, 23), breast, lung, and ovarian (9) carcinoma cells, proliferate within a chamber system. Normal murine hematopoietic cells also grow well within chambers (3, 4, 6), and the effects of various external influences on this growth, such as irradiation (13) and chemotherapy (6, 14), are well studied. Murine leukemia cells also proliferate in a chamber system (10, 25). L1210 murine leukemia has been studied extensively in such a system. Tucker and Owen (30) have grown murine L1210 leukemia cells in the diffusion chamber system on the surface of a chick embryo and, although tumor viability is mildly attenuated by this system, their experiments suggest that rapid cell proliferation may allow quick evaluation of the effects of anticancer agents on tumor cell growth. Cain et al. (7) have treated mice bearing i.p. diffusion chambers containing L1210 leukemia cells with a variety of antitumor agents and found that those agents effective in reducing tumor population in the chamber system also prolonged survival of other mice who had received L1210 cells i.p.

The experiments reported here were designed to assess the reproducibility and predictive value of a diffusion chamber technique (1, 2, 26) for the selection of various regimens of CY capable of prolonging survival of mice bearing L1210 leukemia.

MATERIALS AND METHODS

L1210 Leukemic Cells. Tumor cells were harvested from ascites 5 days following i.p. injection of 2 x 10⁶ L1210 cells into female BD2F1 mice. Cell suspensions were made in Roswell Park Memorial Institute Tissue Culture Medium 1630 (Grand Island Biological Co., Grand Island, N.Y.) containing 15% fetal calf serum, 100 units of penicillin, and 100 mg of streptomycin per ml of medium (Grand Island Biological Co.). Cell number was determined by direct counting of cells in both counting chambers of a hemocytometer.

Host Animals. Host animals were female BD2F1 mice (Charles River Laboratories, Wilmington, Mass.) which were 9 to 11 weeks old at the time of either tumor cell injection or chamber implantation. They were housed in plastic shoe box-type cages and were given Purina laboratory chow and chlorinated tap water ad libitum.

CY Treatment. Survival of animals (5 animals/log concentration of tumor inoculum) following i.p. injection of 10² to 10⁴ L1210 leukemia cells was monitored (Chart 1). The effect on survival of i.p. CY (Mead Johnson, Indianapolis, Ind.) administered in either bolus or divided doses over the range of 12.5 to 250 mg/kg was examined similarly (Chart 2).

Diffusion Chambers. A method similar to that described by Boyum et al. (5) was used. Filter discs (0.22-µm pore size) were cemented to 13-mm Lucite rings (Millipore Filter Corp., Bedford, Mass.) as described by Nettesheim et al. (26). Completed chambers were gas sterilized with ethylene oxide.

Using sterile techniques in a laminar air flow hood, the chambers were moistened in tissue culture media. L1210 leukemia cell suspension (0.1 ml) was injected into each chamber, and the chamber was sealed. Under pentobarbital anesthesia, a chamber was implanted into the peritoneal cavity of each mouse through a midline abdominal incision which was subsequently closed with stainless steel surgical clips. Chambers were harvested (3/day) at different times after implantation from untreated control animals and from animals treated with various doses and schedules of i.p. CY. After removal, the chambers were wiped free of any adherent fibrinous material and agitated for 45 min in Roswell Park Memorial Institute Tissue Culture Medium 1630 containing 15% fetal calf serum and 0.025% Pronase (Sigma Chemical Co., St. Louis, Mo.). They were then rinsed, wiped clean, and opened into 2 ml of fresh media. The number of nucleated cells per chamber was determined (Charts 3 to 5). Tumor cells recovered from chambers implanted in both the untreated and CY-treated hosts were then reinjected into healthy female BD2F1 mice. Mouse survival time was monitored and used to assess the

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3 The abbreviations used are: CY, cyclophosphamide; q.d., daily.
RESULTS

Survival time from i.p. injection of L1210 murine leukemia cells into female BD2F1 mice was linearly related to tumor cell number of the inoculum and was both predictable and reproducible (Chart 1). Treatment with various regimens of CY prolonged survival; single doses prolonged survival to a greater extent than did equivalent divided doses (Chart 2). In particular, CY (250 mg/kg) as a single dose "cured" animals, while CY (62.5 mg/kg) q.d. for 4 days prolonged survival only to 19.0 ± 0.7 (S.E.) days. Cyclophosphamide (50 mg/kg) given 24 hr before tumor cell injection had no effect on survival.

The number of L1210 cells within the chamber rose to $10^8$ cells/cu mm regardless of cell number in the inoculum (Chart 3). Chamber-bearing animals were treated with various doses of CY.
DISCUSSION

The diffusion chamber system is a means of studying the in vivo effect of drug on individual tumor cells and allows monitoring of the effect that various drugs and/or drug regimens may have against tumor cells at intervals after drug administration. Since it is an in vivo system, it offers the opportunity for study of chemotherapeutic agents, such as CY, that require in vivo drug activation (11). Our data on administration of various regimens of CY suggest that the drug effect on tumor cells within the chamber correlates with the drug effect in the whole animal.

CY treatment of host animals reproducibly reduced viability of tumor cells within the implanted diffusion chamber. When these cells were re-injected into healthy animals, a clear-cut decrease on their viability was evident as judged by their reduced ability to recreate tumor and kill the second host (tumorigenicity). This effect is most pronounced if cells are removed from the chamber early after CY treatment. For example, $3 \times 10^5$ counted cells taken from chambers early after i.p. treatment of host animals with CY (62.5 mg/kg q.d. for 4 days) (Table 1) resulted in animal death as if $<10^3$ untreated cells had been injected. This is equivalent to $<1\%$ viability (i.e., capability to recreate tumor) and suggests that most of those cells counted within the chamber were irreversibly damaged and destined to die. In contrast, cells taken from Millipore chambers late (>7 days) after CY treatment showed improved viability (i.e., fewer cells irreversibly damaged), but recovery of viability was not complete. As seen in Chart 5, regardless of the timing of CY treatment, the initial fall and subsequent increase in cell number are similar. Our data therefore concur with those of Cain et al. (7) and suggest that the diffusion chamber system is both reliable and reproducible.

Of utmost importance is the reproducible parallel effect of drug treatment on cells within diffusion chambers and within the whole animal. For example, equivalent single doses of CY cured tumor-bearing animals and tumor cells within Millipore chambers (Charts 2 and 4D). However, equivalent divided doses of CY (i.e., 62.5 mg for 4 days) did not "cure" the tumor-bearing animal or cells within the diffusion chamber. These observations suggest that the Millipore diffusion chamber system may be useful in therapeutic planning.

It has been shown that human tumor cells proliferate within the chamber system (8) and that human leukemic peripheral blood and bone marrow cells from different patients have distinct patterns of chamber growth (9, 17). Assessment of these variable patterns of growth of a single tumor type may prove to be useful.
Treatment of L1210 Leukemia in Diffusion Chambers


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