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THE RESPONSE *IN VITRO*, OF CONTINUOUS CULTURES OF HUMAN LYMPHOBLASTS (CCRF-CEM CELLS) TO CHEMOTHERAPEUTIC AGENTS

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Abstract—A series of nineteen chemotherapeutic agents and one metabolite has been studied for inhibitory activity in continuous suspension cultures of human leukemic lymphoblasts (CCRF-CEM cells), and the resulting ID_{50} data compared with the ID_{50} data derived from previous studies with other kinds of mammalian cells in monolayer cultures.

As adjudged by comparison of ID_{50} data interpolated from dose : response curves in the usual manner, only two chemotherapeutic agents exhibited a greater degree of inhibitory activity for CCRF-CEM cells, and there was little evidence that CCRF-CEM cells are preferentially sensitive to those chemotherapeutic agents useful in the chemotherapy of human leukemia. The difficulties intrinsic in the interpretation of such comparative bioassay data are discussed.

The CCRF-CEM cells were exquisitely sensitive to inhibition by exogenous thymidine, from which some chemotherapeutic advantage may derive. The utility of human leukemic cells in an appropriate bioassay system may reside in the detection of other agents to which such cells may be unusually or uniquely sensitive.

UPON the occasion of the last conference held to consider obstacles to the control of acute leukemia, Bergel[1] commented ". . . A further difficulty confronting the biochemist studying the acute leukemias is the absence of a suitable experimental system in animals that imitates satisfactorily the situation in man. . . ." A corollary may be extrapolated therefrom: would human leukemic cells actively metabolizing and growing *in vitro* provide a better tool for the bioassay of candidate anti-leukemic agents than is provided by the use of other kinds of mammalian cells for this purpose —in other words, would the response of such a bioassay system to chemotherapeutic agents more closely parallel the response of the human disease to the same chemotherapeutic agents.

The successful isolation of human leukemic lymphoblasts[2] (CCRF-CEM cells) in continuous suspension cultures from the peripheral blood buffy coat of a child with lymphosarcoma which had progressed to acute lymphoblastic leukemia provided the opportunity to examine this question. The purpose of the present communication is to describe preliminary experiments in which the response of the CCRF-CEM cells in suspension culture to a selected series of chemotherapeutic agents was

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compared to the response of a variety of other kinds of mammalian cells growing inconventional monolayer cultures.

MATERIALS AND METHODS

The isolation and continuous propagation of the CCRF-CEM cells in suspension culture[2] has been described in detail elsewhere. These cells have retained their classic lymphoblastic morphology even after more than two years in continuous log-phase growth in Eagle's minimal essential medium modified for suspension culture[3] and supplemented with 10% whole fetal calf serum (MEM). They are pseudodiploid,[4] and grow as mono-dispersed suspensions which do not readily adhere to glass (or other) surfaces. The average generation time in culture was *ca.* 40 hr when these experiments were done, and populations of 2–4 $\times$ 10^6 cells per ml can be maintained in continuous log-phase.

Since the CCRF-CEM cells grow properly only in suspension, bioassays were done in "spinner cultures" in screw-capped 125-ml Erlenmeyer flasks containing 50–75 ml of MEM. These cells exhibit a pronounced "population dependence"[5] which severely limits the minimal inoculum which will grow predictably in replicate cultures, thus an inoculum of log-phase cells sufficient to provide a final population of 1·5–2·0 $\times$ 10^6 cells per ml of medium was used in each Erlenmeyer flask culture. Stock solutions of the individual chemotherapeutic agents were prepared in MEM, and diluted with MEM to provide for serial titration at log or half-log concentrations when added to the cultures. All bioassays were in replicate series with appropriate controls, and were incubated at 37° for 48 hr on individual magnetic stirrers operating at *ca.* 60 rpm. Cell counts, using nigrosin exclusion as an index of viability, were done after 24 and 48 hr incubation. The 50 per cent inhibitory dose (ID_{50}) was interpolated from plots of the mean cell counts of replicate cultures at 0, 24, and 48 hr. The methods of bioassay employing monolayer cultures[6–9] of other kinds of mammalian cells have been described in detail elsewhere.

RESULTS

The nineteen chemotherapeutic agents and one metabolite so examined are listed in Table 1, together with their ID_{50} for CCRF-CEM cells in suspension culture and a number of other kinds of mammalian cells in monolayer cultures. Typical dose: response curves for five of these chemotherapeutic agents are illustrated in Fig. 1. It is evident therein that varying degrees of inhibitory activity are readily distinguishable one from the other, even in the relatively simple bioassay system employed in these experiments.

As indicated in Table 1, with three exceptions, there is little evidence that the sensitivity of the CCRF-CEM cells to these chemotherapeutic agents under these conditions of bioassay differs significantly from the sensitivity of other kinds of mammalian cells under the conditions of bioassay imposed by growth in monolayer cultures. Similarly, there is little evidence in these data that the CCRF-CEM cells are preferentially sensitive to those chemotherapeutic agents which are useful in the chemotherapy of human leukemia, as adjudged by comparison of methotrexate, 6-mercaptopurine and vincristine sulfate withactinomyc in D and mechlorethamine HCl (Table 1).

It is of interest to note that as is the case with other kinds of mammalian cells in culture,[10] the CCRF-CEM cells are not inhibited directly by cyclophosphoramide.

On the other hand, cyclohexylammonium hydrogen-N, N-di(2-chlorethyl) phosphorodiamidate,[11] (OMF-59) which is related to cyclophosphoramide, and which, like mechlorethamine HCl, does not require biological "activation" *in vivo*[12] is directly inhibitory; and indeed, the CCRF-CEM cells may be somewhat more sensitive to these agents than are the other kinds of mammalian cells so assayed in monolayer cultures. Similarly, the CCRF-CEM cells appear to be considerably more sensitive to exogenous thymidine and certain related structures (Table 1) than are the other kinds

TABLE 1. COMPARISON OF INHIBITORY ACTIVITY OF SELECTED CHEMOTHERAPEUTIC AGENTS FOR CCRF-CEM CELLS IN SUSPENSION CULTURE AND OTHER MAMMALIAN CELLS IN MONOLAYER CULTURE

Chemotherapeutic agent	ID_{50} in γ/ml*	
	CCRF-CEM cells in suspension culture	Other mammalian cells† in monolayer culture
Methotrexate	0·001–0·01	0·001–0·1
6-Mercaptopurine	1–10	0·1–1
Vincristine sulfate	0·0001–0·001	0·001–0·01
Actinomycin D	0·005–0·05	0·01–0·1
Mechlorethamine HCl	0·005–0·05	0·1–1
Cyclophosphoramide	>500	>1000
Cyclohexylammonium hydrogen-N,N-di (2-chloroethyl) phosphorodiamidate (OMF-59)‡	0·1–1	10–100
5-Fluorouracil	1–10	0·1–1
5′-Fluorodeoxyuridine riboside	0·1–1	0·1–1
2′-Deoxyuridine	10–100	10–100
Azaserine (O-diazoacetyl-L-serine)	0·01–0·1	0·1–1
4,6-Diamino-1-(3′chlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine HCl	0·001–0·01	0·001–0·01
4,6-Diamino-1-(3′,4′-dichlorophenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine HCl	0·001–0·01	0·001–0·01
Chloramphenicol	100–500	10–100
Mitomycin C	0·01–0·1	0·01–0·1
6-Dimethylamino-9-(3′-*p*-methoxy-L-phenylalanylamino-3′deoxy-D-ribosyl) purine HCl (Puromycin dihydrochloride)	0·05–0·5	0·01–0·1
Hydrocortisone	100–500	10–100
Thymidine	0·1–1	>100§
Thymine arabinoside¶	1–10	Not done
Cytosine arabinoside	0·001–0·01	0·1–1

* Log-concentration which caused 50% inhibition of growth. Differences of less than two logs-concentrations are considered to be of doubtful significance.

† KB, HeLa, Chang human liver, Ehrlich ascites, L-1210, P-1534, S-180, MF-929, etc. Bioassay data derived from unpublished studies (these laboratories), and data reported in references 6–9, 12, 24–26.

‡ Known subsequently as Cancer Chemotherapy National Service Center NSC-69945.

§ Includes some cells in suspension culture. Cf. references 15–17, 27.

¶ Kindly supplied by Dr. J. J. Fox, Sloan-Kettering Institute for Cancer Research, New York.

of mammalian cells so assayed in monolayer, and in some instances, suspension cultures.

DISCUSSION

In view of the nature of the results of these preliminary experiments, firm conclusions as to the ultimate utility of human leukemic lymphoblasts as a bioassay tool would be premature. Mammalian cells in suspension cultures have been relatively

little-used in bioassay systems,[13–18] and in view of the obvious differences in the experimental conditions imposed by suspension as opposed to monolayer cultures, it is difficult to assess the significance of apparent similarities (or differences) in sensitivity to a given chemotherapeutic agent. For example, the ID_{50} of most of the chemotherapeutic agents examined herein for the CCRF-CEM cells in suspension culture did not differ significantly from the ID_{50} for other kinds of mammalian cells

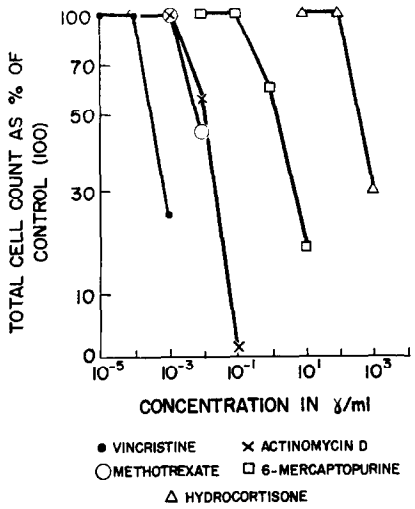

FIG. 1. The inhibitory activity of five representative chemotherapeutic agents for CCRF-CEM cells in suspension culture. Total cell count data based on total viable cell counts after 48 hr exposure to chemotherapeutic agent at 37° (cf. text).

in monolayer cultures when the ID_{50} was extrapolated from dose:response curves in the usual manner. However, the initial cell populations inhibited by this ID_{50} were vastly different; viz. $1{\cdot}5–2{\cdot}0 \times 10^6$ CCRF-CEM cells per ml as compared to $1{\cdot}0 \times 10^5$ cells per ml in the usual monolayer cultures. Thus, it is apparent that the intrinsic sensitivity of human leukemic lymphoblasts can be determined only by direct comparison with other kinds of mammalian cells growing in comparable suspension cultures; and further, by comparison of human leukemic lymphoblasts growing in some kind of modified stationery surface cultures for comparison with other kinds of mammalian cells growing in the usual monolayer culture.

Despite these difficulties in interpretation, it is nonetheless of interest to note the exquisite sensitivity of the CCRF-CEM cells to exogenous thymidine (Table 1). As described in more detail elsewhere,[19] concentrations of exogenous thymidine well below those which have been reported to be non-inhibitory for other kinds of mammalian cells in monolayer or suspension cultures completely inhibit populations of $2{\cdot}5 \times 10^8$ CCRF-CEM cells. As adjudged by incorporation studies with ^{14}C-2-thymidine, this inhibition results from a preferential suppression of DNA synthesis which can be reversed or prevented by deoxycytidine; suggesting that suppression of DNA synthesis may be the result of interference with the synthesis of deoxycytidine nucleotides, as seems to be the case with other kinds of mammalian cells exposed to much higher concentrations of exogenous thymidine.[15, 17, 20] Experiments are now in progress to determine whether some chemotherapeutic advantage might be derived

from this unusual sensitivity to exogenous thymidine. For example, an analog of thymidine which is not readily degraded by thymidine phosphorylase might be of considerable interest as a potential chemotherapeutic agent for human leukemia.

Although the present experiments do not provide the final answer to the corollary question extrapolated from Bergel's comment, it is intriguing to speculate that the use of human leukemic cells in an appropriate bioassay system might result in the detection of other agents to which such cells may be unusually sensitive. The data deriving from such a bioassay system may well prove to be subject to the same limitations in interpretation as are the data deriving from other kinds of mammalian cell bioassay systems,[6–8, 18, 21] but at the same time, such a bioassay system might provide the biochemist with clues indicative of some unusual or unique sensitivity from which some chemotherapeutic advantage may derive.

The CCRF-CEM cells have been successfully implanted and serially transplanted in newborn Syrian hamsters;[22, 23] and in another instance, the peripheral blood buffy coat from another patient with acute lymphoblastic leukemia has been implanted directly and similarly serially transplanted in newborn Syrian hamsters.[23] In either instance, the implanted cells grow as lymphosarcomas which are invasive and metastatic, and which in a significant number of newborn Syrian hamsters, progress to leukemia; thus presenting a striking recapitulation in an experimental animal of the human disease from which they were originally derived. These transplantation studies have provided a new and unique experimental tool which must now be evaluated for its potential utility as a "model" for leukemia in man, which in some respects at least, approximates the needs of the biochemist for a suitable experimental system.

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