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The Use of Human Cancer Cell Lines as a Primary Screening System for Antineoplastic Compounds*

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Abstract—*Exponentially growing cells of human cancer lines have been utilized to investigate the cytotoxic activity of antineoplastic agents. Different cancer cell lines differ greatly in their responsiveness to both clinical and experimental cytotoxic drugs. For example, sensitivities to 5-fluorouracil and cytosine arabinoside ranged over 15- and 30-fold respectively. Cell lines derived from carcinomas were more sensitive to 5-fluorouracil and less sensitive to cytosine arabinoside than was a leukaemic cell line. In general, colon carcinoma lines were most resistant to DNA-intercalating drugs, and a breast carcinoma and leukaemia line most sensitive. In a congeneric series of amsacrine analogues, in vitro patterns of activity against different lines were shown to correlate with activity against the Lewis lung mouse carcinoma in vivo. Results suggest that established cell lines manifest responsiveness to anticancer drugs consistent with that expected from their tumours of origin. This assay is economical, reproducible and convenient, and could be used to complement the human tumour stem cell assay in drug development studies.*

INTRODUCTION

ONE OF of the major problems in experimental cancer chemotherapy is to identify new agents which are selectively toxic towards cancer cells in solid tumours. The selection of tumour cell systems which best reflect the properties of those cells responsible for clinical disease must be of primary importance in the establishment of a reliable screening method for potentially useful antineoplastic agents. In particular, the use of cancer cells of human rather than rodent origin is desirable in view of fears that experimental animal tumours may manifest drug sensitivities which are different to those of human neoplastic cells [1].

Of the many *in vitro* screening techniques which have been described, those employing freshly resected human tumour tissue in clonogenic assays offer the promise of greatest relevance to the problems of clinical cancer [2]. However, they possess the concomitant technical difficulties inherent in the use of clinical specimens, and caution has been urged in the routine application of such procedures [3, 4]. Moreover, cytotoxicity assays featuring survival of clonogenic cells involve extensive technical manipulations which preclude their use when large numbers of compounds are to be assessed for cytotoxic activity. A more facile approach would be to investigate the ability of potential antineoplastic compounds to inhibit exponential growth of continuous cancer cell lines, of which many well-characterized examples are now available [5–12]. For use in prescreening assays for cytotoxic agents, such tissue culture lines would also permit multiple and reproducible comparisons of drug potency over protracted periods of time. A panel of suitable cell lines would also indicate the nature of variation in drug sensitivity between tumour lines of the same histological type.

This report describes our experience in assessing the cytotoxicity of some clinical and

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experimental chemotherapeutic agents against eight human cancer cell lines, and illustrates the wide range of sensitivities exhibited by individual tumour cell lines growing exponentially under standardized conditions *in vitro*. This extends previous observations of heterogeneity of drug sensitivities of cell lines [13–16].

MATERIALS AND METHODS

Cells

The human cell lines used are described in Table 1. Cells were cultured in Alpha MEM (Gibco) supplemented with 10% heat-inactivated foetal calf serum (FCS, Gibco) and glutamine (see Table 1). Some lines were also cultured in the presence of insulin (Novo Actrapid MC) and hydrocortisone (Sigma), as indicated in Table 1. Solid tumour lines were passaged weekly using trypsin (0.1%; Difco) in citrate saline solution (trisodium citrate dihydrate 4.4 g/l, KCl 10 g/l, pH 7.3). At subculture, HCT-8, MCF-7 and T-47D cells were also passed six times through a 26-gauge hypodermic needle in order to produce a suspension comprising mainly single cells. Cells were regularly tested for the presence of mycoplasma by the method of Chen [17]. As an additional precaution against low-level contamination, cells were cultured in the absence of antibiotics at all times.

Cytotoxicity assays

The cytotoxic activity of a drug is described in terms of the concentration required to decrease cell number to 50% of control values (the ID_{50}) in exponentially growing cultures exposed to the drug for 3–5 cell doublings.

To establish cultures, 5×10^3 cells (intact and refractile by phase-contrast microscopy) were added in 0.9 ml of culture medium into the wells of 24-well tissue culture dishes (Nunc) and cultured at 37°C in a humidified atmosphere of 5% CO_2 in air. The culture dishes were enclosed in a plastic bag containing water to prevent evaporation from the cultures, which otherwise proved to be a serious problem in slower growing cell lines. Cells were cultured for two days to allow them to resume exponential growth, after which drug was added in 0.1 ml of culture medium to encompass a 16-fold concentration range (five 2-fold dilutions, each concentration in duplicate). Cultures were incubated for another 4–7 days (Table 1), depending on the growth rates of the individual lines.

To estimate cell number at the termination of the culture period, Jurkat cells were passed 6 times through a 20-gauge needle, the contents of each well added to 9.0 ml of NaCl solution (9 g/l) and cells enumerated with an electronic particle counter (Counter Electronics). Culture supernatants of other lines were aspirated, monolayers

Table 1. *Human cancer lines and their growth characteristics*

Cell line	Histologic type	Ref.	Modal chromosome No.	Relative DNA content *	Medium supplements	Doubling time (hr) †	Duration of drug assay (days)
HCT-8	Colon adenocarcinoma	11	48	48.3	FCS 10% glutamine 2mM	15	6
HT-29	Colon adenocarcinoma	7	hypo- to hyper-triploid	72.6	as for HCT-8	24	7
LoVo	Colon adenocarcinoma	6	49	51.4	as for HCT-8	15	6
MCF-7	Breast, infiltrating ductal carcinoma	10	88	80	FCS 10% glutamine 6 mM insulin 10 μg/ml	32	8
MDA-MB-231	Breast, adenocarcinoma	5	69–70	60.4	FCS 10% glutamine 2 mM insulin 2 μg/ml hydrocortisone 200 ng/ml	24–30	7
T-47D	Breast, infiltraing ductal carcinoma	8	66	65	as for MDA-MB-231	37	9
MM-96	Malignant melanoma	12	47	53.2	as for MDA-MB-231	13	6
Jurkat‡	T cell leukaemia	9	?	88.7	as for HCT-8	19	6

*Arbitrary scale, with human diploid fibroblasts equal to 46.
†Population doubling time during exponential growth phase.
‡Jurkat cells designated JM in ref. [9].

rinsed once with phosphate-buffered saline (NaCl 8 g, KCl 0.2 g, anhydrous KH_2PO_4 0.2 g, anhydrous Na_2HPO_4 1.15 g, $CaCl_2$ 0.1 g, $MgCl_2.6H_2O$ 0.1 g, in 1 l of water), trypsinized (10 minutes at 37°C in 0.1% trypsin:citrate saline) and disaggregated by passage through a 26-gauge needle. The contents of each well were diluted in saline and the cells enumerated as described aboved.

Cytotoxic drugs

Sources are as follows: doxorubicin (Farmitalia), daunorubicin (May and Baker), actinomycin D (Sigma). Amsacrine analogues and nitracrine were synthesized in this laboratory. Other agents were a gift from the Warner-Lambert Company, Ann Arbor, MI, U.S.A.

Drugs were dissolved in 50% v/v aqueous ethanol to a final concentration of 1 mM, except for methotrexate, which was first dissolved in a small volume of dimethylsulphoxide before adding 50% ethanol to the final required concentration, and *cis*-diamminedichloroplatinum, which was dissolved in phosphate-buffered saline. The amount of ethanol in culture never exceeded 0.25% v/v, a concentration shown to have no effect on cell growth.

Flow cytometry

An Ortho Instruments Model ICP 22A analyser was used to determine DNA content and distribution in single-cell suspensions. Cells were permeabilized withTriton X and stained with diamidinophenylindole following the method of Taylor [18]. Pigeon erythrocytes were utilised as an internal standard, and the machine was calibrated using the coincidence peaks of pigeon erythrocyte suspensions.

RESULTS

Characterisation of cell lines

The cell lines used, their histological type and the reference describing their establishment are listed in Table 1. Published modal chromosomal numbers are also indicated, and range from 47 to almost tetraploid. Cell lines have been characterized with respect to their DNA contents by flow cytometry, and the relative DNA contents are also shown, illustrating that each line is distinguishable by this property and that it correlates with the chromosome number ($r = 0.964$ for six cell lines). Doubling times differ substantially between lines. The colon lines and MM-96 appear to grow faster than expected from published values, and this may be a function of growth conditions (growth medium or serum supplement). To compensate partially for the diversity of growth rates, the drug sensitivity assays were performed over 6–9 days (4–7 days drug exposure), depending on the cell line.

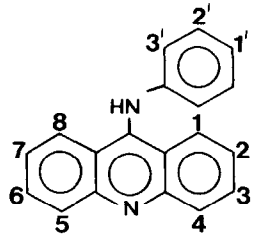
Where multiple ID_{50} determinations were performed for the same agent, coefficients of variation (CVs) were generally less than 40%. The ranges of CVs are 3–46% (Table 2) and 5–40% (Table 3 excluding amsacrine). The mean CV of all data in these tables is 19%.

Table 2. Cytotoxic activities of 13 clinical agents to one murine and 8 human cancer cell lines

	ID_{50} (nM)								
	Colon carcinoma			Breast carcinoma			Melanoma	Leukaemia	
Compound	HCT-8	HT-29	LoVo	MCF-7	MDA-231	T-47D	MM-96	Jurkat	L1210
Antimetabolites									
Cytosine arabinoside (AraC)	46(2)*	235(3)	95	64(2)	18	100	17(2)	6.4	28
5-Fluorouracil (5FU)	1730(2)	520(3)	390	1980(2)	3800	790	6100(2)	5100(2)	1300
Methotrexate (MTX)	13	14	11	4	>44	15	20	9.2	18
Agents which bind covalently to DNA									
Nitrogen mustard	500(2)	400(2)	790	990	310	280	240	240(3)	304
cis-Platinum	1400(2)	760(2)	800	670	450	1300	670	380(3)	460
Agents which intercalate into DNA									
Actinomycin D	0.98	0.47	0.33	0.18	0.10	0.22	0.47	0.18	0.33
Doxorubicin	63	25(3)	13	13	20	2.1	6.3	9.2	30
Daunorubicin	220	83(3)	75	56	73	22	48	38	20
Ametantrone	230	190	200	49	91	25	35	18	52
Bisantrene	260	72	74	25	27	19	23	6.4	15
Mitoxantrone	13	12	6.9	4.4	2.8	0.82	2.0	1.0	0.75
Amsacrine	70(9)	72(8)	47(4)	79(5)	77(3)	28(8)	50(7)	12(4)	32
Nitracrine	4.4	5.3	8.6	3.2	5.0	2.9	6.6	6.4	22

*Means; values in parentheses indicate the number of independent determinations if greater than 1.

Table 3. Cytotoxic activities of amsacrine derivatives ID_{50} *values* (nM)



Compound	Substituents	Ref.	Colon carcinoma HCT-8	HT-29	LoVo	Breast carcinoma MCF-7	MDA-231	T-47D	Melanoma MM-96	Leukaemia Jurkat	L1210
1	1'NH_2	30	570	870(2)*	700	700	1000	510(2)	730(2)	340	470
2	1'$NHSO_2$ Ph NH_2†	30	8.5	8.8(3)	2.9	6.6	5.8	1.8	3.9(2)	0.95	0.8
3	1'$NHSO_2CH_3$	30	210(3)	520(2)	250	370	280	130(3)	300	38	38
4	1'$NHSO_2CH_3$ 3'OCH_3 ‡	31	70	72	47	79	77	28	50	12	32
5	1'$NHSO_2CH_3$ 3'OCH_3 3$NHCH_3$	31	58(3)	22(3)	7.6	3.6	18	1.2(2)	5	2.9	6.0
6	1'$NHSO_2CH_3$ 3'OCH_3 3$NHCO_2CH_3$	31	220(2)	140	52	66	52	7.5	29	11	41
7	1'$NHSO_2CH_3$ 3'OCH_3 3Br	31	19(3)	13(3)	9.8	16	19	6	13	3.6	49
8	1'$NHSO_2CH_3$ 3'OCH_3 4CH_3	31	43(2)	38	21	43	50	16	30(2)	7.3	30
9	1'$NHSO_2CH_3$ 3'OCH_3 4CH_3 5$CONHCH_3$	29	25(2)	20(3)	12	18	31	6.7(2)	16(2)	3.6	38

*Numbers in parentheses: number of determinations.

†*para*-Aminobenzenesulphonamide derivative.

‡Amsacrine, data from Table 2.

A comparison of the responsiveness of 8 human cancer lines to clinical and experimental cytotoxic agents

The ID_{50} values for cytosine arabinoside (AraC), 5-fluorouracil (5FU) and methotrexate (MTX) for each of the eight cell lines are shown in Table 2. Values for cultured L1210 murine leukaemia cells are also included for comparison. Since a different medium was used for culture, values are not directly comparable. It is evident that the cell lines display unique patterns of sensitivity, with no one line appearing consistently more sensitive or resistant.

For AraC, the T cell leukaemia line shows greatest sensitivity. Two of the solid tumour lines are also markedly more sensitive than the other carcinoma lines. The most resistant line, HT-29, requires at least 30 times the concentration of AraC to demonstrate the same degree of inhibition, as do Jurkat leukaemia cells. In contrast, Jurkat and MM-96 are the least responsive lines to 5FU. This drug is the only one identified where all the carcinoma cell lines are more responsive than the leukaemia. There is a wide range of sensitivities between lines (15-fold) for this agent. MTX is a uniformly potent cytotoxic agent for all cell lines with the exception of MDA-MB-231.

The effects of nitrogen mustard and *cis*-diamminedichloroplatinum on the panel of lines has been investigated, and ID_{50} values are presented in Table 2. In sharp distinction to their responses to antimetabolites, the cell lines do not vary widely in their sensitivities to these two agents, the ID_{50}s falling within a 4-fold range in each case.

The cytotoxic effects of a selection of DNA-binding agents are shown in Tables 2 and 3. In contrast to their responses to antimetabolites, the different human lines manifest consistent patterns of responsiveness to these drugs. HCT-8 and HT-29 cells are generally most resistant, whereas T-47D and Jurkat cells are most responsive. However, as with the antimetabolites, the panel of cell lines shows a wide range of sensitivities to most of these agents. Three broad classes of drug can be described in terms of their effects on the panel of cells:

(a) Drugs against which the different cell lines show a wide (at least 10-fold) range of sensitivity. These include actinomycin D, the anthracyclines, ametantrone, bisantrene, mitoxantrone (Table 2) and several amsacrine analogues (compounds 2, 5 and 6 in Table 3). HCT-8 is invariably most refractory, followed by another colon line, HT-29. T-47D is the most sensitive of the carcinomas, with ID_{50} values generally similar to those of Jurkat leukaemia cells.

(b) Drugs against which the different lines show a moderate (5- to 10-fold) range of responsiveness. This class includes amsacrine and most of the amsacrine analogues (Tables 2 and 3, together with many others, not shown). ID_{50} values of HCT-8 cells are similar to most of the other carcinoma lines, and T-47D cells remain the most sensitive of the solid tumour-derived lines. However, Jurkat T cell leukaemia cells are now killed preferentially relative to T-47D by compounds of this group.

(c) The third class, exemplified only by nitracrine (Table 2), shows minimal discrimination between human cancer cell lines, a property it shares with nitrogen mustard and *cis*-diamminedichloroplatinum. Of the DNA-binding compounds, nitracrine is unique in that it is not more cytotoxic for the leukaemia cells than for carcinoma cells, and indeed with 5FU alone shares the property of being more active against both HCT-8 and HT-29 than Jurkat cells.

Results in Table 3 illustrate the usefulness of cell lines in drug analogue development studies. Small alterations in chemical structure can markedly alter cytotoxic activity. Thus the addition of a 3′-methoxy group to compound 3 to yields amsacrine results in a several-fold increase in activity. Similarly, substitutions with 4-methyl (compound 8) and 5-$CONHCH_3$ (compound 9) moieties progressively potentiate cytotoxicity against all cell lines. In contrast, substituted amines at position 3 (compounds 5 and 6) are ineffective or unfavourable against some lines (HCT-8, HT-29) but highly favourable against others (T-47D).

DISCUSSION

The method described in this report for evaluating the activity of cytotoxic drugs *in vitro* incorporates the desirable features of technical simplicity, reproducibility and an endpoint relevant to drug sensitivity of human tumour cells. The assay can be applied to most antitumour drugs including antimetabolites, agents which modify DNA covalently and those which intercalate into DNA (Tables 2 and 3). Experiments (not shown) with DNA-binding agents have shown that as drug concentration is increased, the clonogenic potential is lost, demonstrating that the ID_{50} value reflects cytotoxicity and not merely a drug-mediated cytostatic effect. A number of human solid and haematological tumours, including those with doubling times of over 30 hr (Table 1), have proved to be satisfactory as target cells in the assay. By means of flow cytofluorimetry, cell lines are readily monitored for variation between labora-

tories, for karyotypic drift during prolonged passaging and for contamination by other cells.

This study emphasizes the great variation (10-to 40-fold) in the intrinsic responsiveness of cell lines within a broad group of clinically used agents, and also within a closely related series of amsacrine derivatives. Sensitivity is not a simple function of population doubling time (Table 1). For the antimetabolites, variations in intracellular target enzyme concentrations are probably responsible for changes in sensitivity, whereas for DNA-binding agents inward and/or outward transport mechanisms may be important [19].

Among the clinical agents, there is evidence that *in vitro* selectivity in this test is related to the clinical spectrum of activity. AraC and amsacrine have highly selective antileukaemic activity in the clinic [20, 21]. Both these compounds are more toxic for Jurkat leukaemia cells than for any of the carcinoma-derived lines (Table 2). Conversely, 5FU is widely used in the treatment of carcinomas, and is the only agent used in this study which is more cytotoxic for all the carcinoma-derived lines than for the leukaemia line. The cytotoxicity of MTX for most cell lines at similar concentrations is consistent with its broad spectrum of clinical usefulness. The DNA-binding drugs (in particular ametantrone, bisantrene and mitoxantrone, to some extent actinomycin D and the anthracyclines) are more cytotoxic for the breast than the colon carcinoma lines (Table 2), in accord with the known resistance of colon cancer to chemotherapy [22]. Nitracrine, which behaves as an alkylator [21, 23] in addition to possessing the properties of an intercalating drug, is at least as toxic to carcinoma lines as it is to Jurkat cells (Table 2), consistent with its reported activity against mammary and ovarian carcinoma and lack of haematological toxicity [23]. This compound is of current interest in this laboratory because of its selective toxicity towards hypoxic cells [24].

Clonogenic assays, in which cells are exposed to a cytotoxic agent and then cultured in order to count the number of surviving (clonogenic) cells, are of use in the prediction of clinical activity [25, 26]. The relative cytotoxicities of four DNA-binding compounds (mitoxantrone, doxorubicin, ametantrone and bisantrene) are very similar when determined by the assay system described above and by a clonogenicity assay [27]. Thus for this class of compounds the ID_{50} procedure may correlate well with the more technically demanding clonogenic assay.

For each experimental agent in Table 3, relative cytotoxicities against the different cell lines *in vitro* have been compared with activity against an experimental solid tumour in mice. The anti-tumour activities of compounds 4–8 (Table 3) against the Lewis lung carcinoma have previously been published [28]. ILS_{max} (maximal increase in life extension) values for compounds 1, 2, 3 and 9 have been determined using the same protocol to be 117, 8, 55 and 167%. When the ratio of ID_{50} values of more resistant (HCT-8 or HT-29) to more sensitive (T-47D or Jurkat) cell lines are plotted vs experimental antitumour activity (Fig. 1) it can be seen that those compounds with the highest *in vivo* activity are relatively more active against the resistant cell lines (have lower ratios), whereas those which are inactive *in vivo* are relatively less active against the resistant lines (have higher ratios). These relationships also predict to a large extent the inactivity of doxorubicin, daunorubicin, ametantrone and mitoxantrone in the Lewis lung assay [28], which are also plotted in Fig. 1.

Current extensions to the procedure which are under investigation are the utilisation of further human leukaemia cell lines in order to ascertain the range of leukaemia cell sensitivities, the automation of the method for large numbers of samples and the adaptation of the method to measure the effect of 1-hr drug treatments of cells. The correlations found so far between ID_{50} ratios and activity towards experimental solid tumours (ref. [28] and unpublished results) suggest that, at

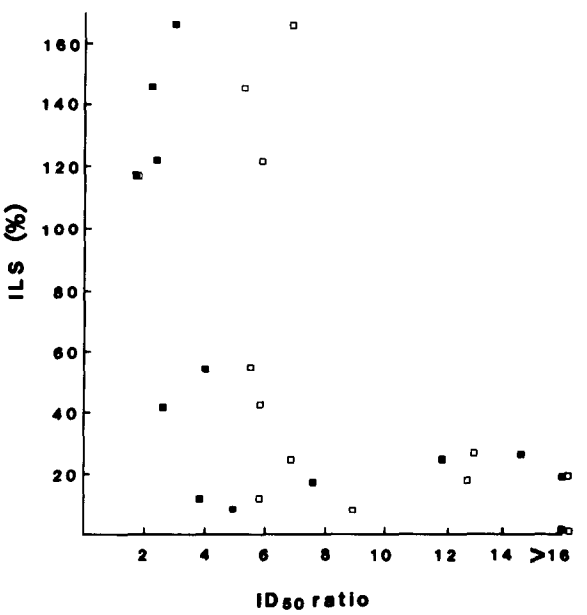


Fig. 1. Relationship between the maximal increase in life-span of mice with the Lewis lung tumour and the ratios of ID_{50} *values for different cell lines. Mice were injected intravenously with* 10^6 *tumour cells on day 0 and intraperitoneally with drug on days 5, 9 and 13 [28].* ID_{50} *ratios are calculated from Tables 2 and 3 for amsacrine derivatives, doxorubicin, daunorubicin, ametantrone and mitoxantrone.* ■ ID_{50} *(HT-29)/*ID_{50} *(T-47D);*□ ID_{50} *(HCT-8)/*ID_{50} *(Jurkat).*

least for the classes of DNA-binding agents currently under study, highly useful data can be obtained with this technique. Compound 9 (Table 3), as CI-921 (NSC 343 499) [29], is a candidate compound for clinical trial.

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