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Comparison of *In Vivo* and *In Vitro* Drug Sensitivities of Lewis Lung Carcinoma and P388 Leukaemia to Analogues of Amsacrine*

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Abstract—*The activity of several clinical agents (5-fluorouracil, methotrexate, adriamycin, daunorubicin, mitoxantrone and amsacrine) and of a number of analogues of amsacrine, including the 4-methyl,5-(N-methyl)carboxamide derivative (CI-921) which is at present in clinical trial, has been compared* in vivo *against Lewis lung carcinoma (LL) and P388 leukaemia in mice, and against corresponding cell lines in cell culture. All derivatives were active against i.p. inoculated P388 leukaemia whereas only some were active against i.v. inoculated LL cells. The relative* in vitro *activities in the two cell lines, as measured by growth inhibition (IC_{50}) assays, varied from equitoxic to 26-fold more active with P388 cells than with LL cells. The* in vivo *activity of these drugs against i.v. inoculated LL relative to i.p. inoculated P388 could be predicted with a high degree of significance from the ratio of* in vitro *activities in the 2 cell lines. However, this correlation did not appear to reflect cell line selectivity alone, since, when P388 cells were inoculated i.v. rather than i.p., drug sensitivity closely matched that of the LL tumour. This observation suggests a dominant role for pharmacological variables in determining the* in vivo *activity of amsacrine analogues, and underlines the importance of standardising tumour site in the determination of antitumour spectrum. Nevertheless, the correlation of selective* in vitro *toxicity for cultured LL cells with high activity against remotely implanted tumours demonstrates the utility of* in vitro *tests in identifying amsacrine analogues with improved clinical potential.*

INTRODUCTION

THE WIDE variety of drug sensitivities displayed by different tumour cell line represents a major challenge for chemotherapy. This variability in drug sensitivity creates a need for *in vitro* screens that will predict *in vivo* activity against individual tumours. It also presents special problems for the development of new anticancer drugs, most of which are selected on the basis of activity against a very small panel of experimental tumours. The current study is part of an investigation of screening strategies which seeks to maximise information from drug sensitivity measurements with cultured cell lines derived from the *in vivo* tumours. The specific hypothesis tested here is that it might be possible to predict the *in vivo* activity of a drug against a new tumour *Y* relative to the primary screening tumour *X* on the basis of the relative *in vitro* drug sensitivities of cell lines derived from *X* and *Y*.

The present study uses the P388 murine leukaemia as an example of a highly drug sensitive primary screen, and the Lewis lung carcinoma (LL), which responds to most alkylating agents and to some antimetabolites, but is unresponsive to most DNA binding agents [1, 2] as a more refractory solid tumour. The recently-introduced clinical anti-leukaemia agent amsacrine (see ref. [3] for review and Table 1 for structure) has only marginal activity against LL, whereas some derivatives of amsacrine, including the analogue CI-921 (NSC 343 499) [4] which is currently undergoing clinical trial, are highly effective, with therapeutic activity comparable to cyclophosphamide. It has not been determined whether the divergence in activity between these 2 tumours reflects differences in intrinsic cellular sensitivity or whether it arises from factors such as cytokinetics or drug distribution. However,

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it is known that certain derivatives of amsacrine have a cell line specificity which is different from that of amsacrine [5, 6]. We have therefore compared the *in vitro* activity of a number of amsacrine derivatives, as well as that of a small number of clinical agents, against cell lines derived from the P388 leukaemia and the LL tumour. We have examined the contribution of changes in *in vitro* selectivity to the relative activities of these drugs against the corresponding tumours *in vivo*.

MATERIALS AND METHODS

Materials

All amsacrine congeners were synthesised in this laboratory [4, 7, 8] and were pure at the time of the study as judged by thin-layer chromatography.

Cell lines

Two mouse tumour lines, the P388 leukaemia and the Lewis lung (LLAK) tumour, were obtained in 1977 as frozen stocks from the Developmental Therapeutics Program, Divison of Cancer Treatment, National Cancer Institute, U.S.A. The *in vitro* Lewis lung line (LLTC) was obained in 1981 from Dr. R.C. Jackson at the Warner-Lambert Company, Ann Arbor, Michigan, and was developed from LL at the Southern Research Institute, Birmingham, Alabama [9].

Cell culture

P388 cells from intraperitoneal ascites in DBA/2J mice were cultured in RPMI 1640 medium containing heat-inactivated foetal calf serum (10% v/v), mercaptoethanol (50 μM), penicillin (100 U/ml) and streptomycin (100 μg/ml) as previously described for L1210 cells [6, 10]. In some cases the medium contained 50 μM sodium ascorbate in addition to the above components. Cells were adapted to culture for at least 3 weeks before use in growth inhibition studies, and were then maintained for up to 12 weeks by subculture to 3×10^4 cells/ml in 25 cm^2 T-flasks (doubling time 12 hr). Cultures of LLTC cells were initiated from frozen stocks every 10 weeks using Alpha MEM containing serum and antibiotics as above, and were passaged by subculture to 10^4 cells/ml in 25 cm^2 T-flasks every 3–4 days (doubling time 13 hr). Cells had a limited capacity to flatten on the substrate, and tended to detach readily. Cells were trypsinised (0.7% Difco Bacto trypsin in 0.015 M trisodium citrate, 0.134 M KCl, pH 7.2) for 10 min at 37°C and single cell suspensions were prepared by vigorous pipetting. Both P388 and LLTC cell lines were free of mycoplasma as judged by cytochemical staining [11].

In vitro *growth inhibition (IC_{50} assays)*

A P388 cell suspension was diluted to 3×10^4 cells/ml in growth medium with antibiotics. 1.0 ml samples were dispensed into 24-well trays (Nunc) and incubated at 37°C under 5% CO_2. After 2 hr, drugs were added to cultures as 50% ethanol–water solutions (2 μl/well). Cultures were gently mixed and incubated for 70 hr before determination of cell density with an electronic cell counter (Coulter Electronics). Control cell density was typically 10^6/ml. Each test included 5 2-fold drug dilutions in duplicate and 4–8 control cultures. The IC_{50} was defined as the drug concentration required to reduce the cell density to 50% of that of the controls.

An analogous determination of growth inhibitory potency was performed with LLTC cells. Cultures were established by seeding trypsinised log-phase LLTC cells into 24-well trays (10^4 cells/ml, 0.5 ml/well). After incubation for 24 hr to allow resumption of log-phase growth, drugs were added in growth medium (0.5 ml). After incubation for 48 hr, the monolayers were rinsed gently with PBS (NaCl 8 g/l, KCl 0.2 g/l, KH_2PO_4 9.2 g/l, Na_2HPO_4 1.15 g/l, $CaCl_2$ 0.1 g/l, $MgCl_2$ 0.1 g/l) and trypsinised for 10 min at 37°C in 0.1% trypsin solution (1 ml). A single cell suspension was prepared by vigorous pipetting, and was diluted for cell counting in 0.9% NaCl containing 1% foetal calf serum. Cell densities were measured as above. The control cell density was typically 1.5×10^5/ml.

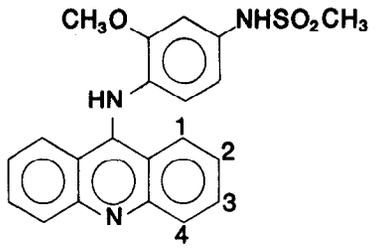
Growth of tumours in mice

P388 cells were passaged weekly in DBA/2J female mice by i.p. injection of 10^6 cells. Cells were removed from carrier mice by peritoneal washing with PBS. LL tumours were passaged every 14 days in C57BL/6J mice by s.c. injection of 10^5 cells. Solid tumours were disaggregated by passage through a 100-μm nylon monofilament mesh and a 26-gauge needle and large nucleated cells were counted in a haemocytometer. Further details have been published previously [2].

Assessment of antitumour activity

Antitumour effect *in vivo* was determined using life extension assays for both P388 leukaemia and LL lines as previously described [2]. Tests were also carried out in which P388 cells were injected i.v. B6D2F1 hybrid mice (DBA/2J male × C57BL/6J female) were inoculated i.p. or i.v. with 10^6 P388 cells or i.v. with 10^6 LLAK or LLTC cells in 0.2 ml PBS on day 0. Drugs were administered i.p. to groups of 6 mice as solutions in 0.1 ml of 30% (v/v) aqueous ethanol on days 1, 5, 9 (P388) or days 5, 9, 13 (LL) unless otherwise indicated. Clinical drugs were dissolved in 5% dextrose–water and given as above, except for cyclophosphamide which was reconstituted directly from the clinical vial (which contained NaCl) by addition of water, and injected as a fresh solution as a single dose. In each test a dose–response profile was determined with at least 3 dose levels at 1.5-fold increments, for each

Table 1. In vitro *growth inhibition (IC_{50}) data for clinical agents and amsacrine derivatives*



No.	Compound	IC_{50} values* P388 −ascorbate	P388 +ascorbate	LLTC (+ascorbate)	IC_{50} ratio
1	5-Fluorouracil	1130 ± 180	1130 ± 50	1150 ± 180	1.02
2	Methotrexate	8.2 ± 0.4	5.2 ± 0.3	41 ± 10	7.9
3	Adriamycin	15 ± 2.5	17.3 ± 1.9	26 ± 4.8	1.5
4	Daunorubicin	24 ± 7.4	11.3 ± 2.1	51 ± 9	4.5
5	Mitoxantrone	0.8 ± 0.05	0.7 ± 0.13	4.2 ± 0.6	4.9
6	Amsacrine	23 ± 2.6	12.5 ± 0.7	26.5 ± 1.3	2.1
7	3-CH_3	7.3 ± 0.4	1.7 ± 0.4	7.5 ± 0.5	4.4
8	3-OCH_3	20 ± 3.0	2.2 ± 0.2	15.5 ± 2.5	7.0
9	3-Cl	19.3 ± 3.9	6.7 ± 1.0	16.5 ± 3.5	2.5
10	3-Br	11.3 ± 1.6	4.2 ± 0.2	10.5 ± 2.1	2.5
11	3-NH_2	16 ± 1.0	2.1 ± 0.4	54 ± 12	25.7
12	3-$NHCH_3$	9.2 ± 1.9	1.8 ± 0.1	23 ± 6	12.8
13	3-$NHCOCH_3$	55 ± 26	5.0 ± 0.7	100 ± 10	20.0
14	3-$NHCOOCH_3$	26 ± 6	7.2 ± 1.5	53 ± 7	7.4
15	3-NO_2	22 ± 5	6.7 ± 3.2	22 ± 3	3.3
16	3-$CONHCH_3$	116 ± 30	37 ± 5	370 ± 47	10.0
17	4-CH_3	9.6 ± 2.8	11.1 ± 0.2	14.8 ± 3.9	1.3
18	4-OCH_3	32 ± 2	3.1 ± 0.1	9.2 ± 0.2	3.0
19	4-$CONHCH_3$	60 ± 8	20 ± 0.3	46 ± 6	2.3
20	4-CH_3,5-$CONHCH_3$	20 ± 6	4.9 ± 0.8	12.6 ± 1.2	2.6

*Mean nanomolar concentration ± standard error for 2–10 determinations.

drug, to bracket the optimal dose. Deaths were recorded daily and long-term survivors were recorded on day 50 (P388) or day 60 (LL). Mean percentage increases in life-span with the exclusion of long-term survivors, were calculated with respect to the mean control life-span in each experiment (20–30 control mice). In almost all cases, LL results represent the average of 2 separate determinations, most of which have been published previously [2].

RESULTS

In vitro *relationships for amsacrine derivatives*

The drug sensitivities of LLTC and P388 cell lines were compared under culture conditions which were as similar as possible for the two lines. However, optimal growth rates (doubling time 12–13 hr for both cell lines) were obtained in different media (Alpha-modified MEM and RPMI 1640 respectively). Studies on the influence of various components of the growth medium on drug toxicity have indicated that compounds in the amsacrine series are stabilised in culture by the presence of ascorbate, which presumably reduced the quinone diimine oxidation products back to the starting material (G.J. Finlay, personal communication, and ref. [5]). Since RPMI 1640 does not contain ascorbate while Alpha-MEM does, IC_{50} values were compared in RPMI 1640 with and without ascorbate (Table 1). IC_{50} values for P388 cells in the absence of ascorbate were similar to those previously reported for L1210 leukaemia cells [6]. Ascorbate (50 μM) exerted a major protective effect on most of the amsacrine analogues, as indicated by decreases in IC_{50} values (Table 1). Comparisons between the 2 cell lines were therefore based on values in media containing ascorbate.

A group of acridine-substituted derivatives of amsacrine, including the clinical candidate CI-921, was chosen to include compounds ranging from highly active to inactive (ILS less than 40%) in the LLAK life extension assay. A group of clinical cytotoxic agents was also included for comparison. All compounds were active against P388 leukaemia

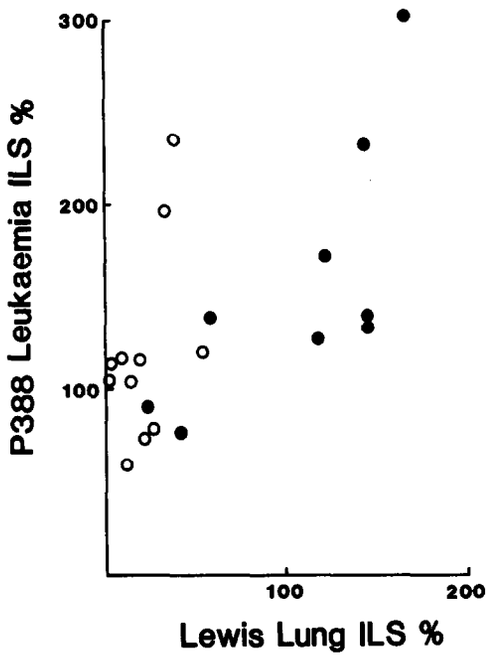


Fig. 1. Plot of percentage increase in life-span (ILS) induced by compounds in Table 2 using i.p. inoculated P388 leukaemia and the i.v. inoculated Lewis lung (LLAK) tumour. Those compounds with a ratio of IC_{50} values (LLTC/P388) of 3 or less are shown as closed circles and the remainder are shown as open circles.

in vivo. In culture the ratios of IC_{50} values (LLTC/P388) varied from 1.02 to 26, indicating marked differences in cell line selectivity.

Relationships between in vivo *and* in vitro *activity*

The maximal life extensions achieved at the optimal dose for each compound is shown for P388 and LLAK in Fig. 1 and Table 2. Life extensions do not correlate with logarithmic IC_{50} values alone. However, it is noteworthy that all compounds with an IC_{50} ratio of greater than 3.0 are inactive against LLAK, and that the remaining compounds, with the exception of adriamycin, are active (Fig. 1). The predictive value of the *in vitro* assays was tested by determining whether the maximal increase in life-span (ILS) for LLAK could be described as a function of P388 activity and the IC_{50} ratio for this set of compounds. Regression analysis demonstrated that the 2 life extension assays were correlated ($r = 0.61$; $P < 0.05$) but that the logarithmic IC_{50} ratio entered the correlation ($F = 8.1$; $P = 0.01$) to provide a highly significant final equation ($r = 0.78$; $P < 0.001$).

$$\text{ILS(LLAK)} = 0.50(\pm 0.14)\text{ILS(P388)} - 72.0(\pm 22.6)\log(\text{ratio}) + 35.7$$

Table 2. In vivo *antitumour activity in P388 leukaemia and Lewis lung carcinoma: clinical agents and amsacrine derivatives*

		P388 leukaemia				LLAK	
		i.p.		i.v.		i.v.	
No.	Compound	OD* (mg/kg)	ILS† (%)	OD (mg/kg)	ILS (%)	OD (mg/kg)	ILS (%)
1	5-Fluorouracil	65	139(1)	45	70	65	59
2	Methotrexate	100	235(2)	45	83	100	39
3	Adriamycin	5.9	90	5.9	57	2.6	25
4	Daunorubicin	2.7	60	3.9	0	3.9	12
5	Mitoxantrone	3.3	79(1)	2.6	70	3.9	27
6	Amsacrine	13.3	78	13.3	25	13.3	42
7	3-CH_3	10.0	120(1)	13.3	25	13.3	54
8	3-OCH_3	8.9	196(4)	13.3	18	13.3	34
9	3-Cl	20	232	20	78	30	144(4)
10	3-Br	20	133(2)	20	118	20	146(2)
11	3-NH_2	8.0	116	8.9	10	5.9	20
12	3-$NHCH_3$	3.0	117	5.9	20	2.7	10
13	3-$NHCOCH_3$	13.3	105	20	25	20	14
14	3-$NHCOOCH_3$	5.9	105	8.9	5	3.9	1
15	3-NO_2	5.9	73	8.9	12	5.9	12
16	3-$CONHCH_3$	30	115(1)	45	22	45	4
17	4-CH_3	20	172(3)	20	90	20	122
18	4-OCH_3	30	139(5)	30	76(1)	45	145(1)
19	4-$CONHCH_3$	30	127	45	40(1)	45	119(2)
20	4-CH_3,5-$CONHCH_3$	20	300(3)	20	152	30	167(3)

*Optimal dose (mg/kg/dose; days 1, 5, 9 for P388, days 5, 9, 13 for LLAK).

†Increase in life-span at optimal dose. Values in parentheses represent numbers of long-term survivors (averaged for multiple determinations) from groups of 6 mice.

Table 3. In vivo *activity of clinical and experimental drugs towards LLAK and LLTC*

No.	Compound	Schedule: Day 1,5,9 OD*	ILS† LLTC	ILS LLAK	Schedule: Days 5,9,13 OD*	ILS LLTC	ILS LLAK	IC_{50} Ratio
0	Cyclophosphamide†	225	96	200	—	—	185	—
1	5-Fluorouracil	65	16	67	—	—	59	1.0
6	Amsacrine	13.3	14	38	13.3	9	42	2.1
7	3-CH_3	13.3	3	66	13.3	21	54	4.4
9	3-Cl	20	41	183	20	67	145	2.5
10	3-Br	20	68	247	20	65	146	2.5
12	3-$NHCH_3$	1.8	1	11	—	—	10	13
15	3-NO_2	5.9	0	0	—	—	12	3.3
17	4-CH_3	20	62	90	13.3	37	122	1.3
18	CI-921	30§	73	168	30	105	167	2.1

*Optimal dose (mg/kg/dose; data for the LLTC tumour).
†Percentage increase in life-span at optimal dose.
‡Single dose on first day of schedule.
§In multiple experiments the optimal dose varied between this value and one 1.5-fold lower.

Results with intravenously inoculated P388

The above results suggest that the *in vitro* selectivity of drugs in continuous exposure IC_{50} assays correlate with their relative *in vivo* activity. As a further test of this hypothesis, the activity of the same set of drugs was evaluated against P388 cells which had, like LLAK, been inoculated i.v. so that the site of tumour growth was remote from the site of drug injection (Table 2). As expected, activity was attenuated compared to the results with i.p. injected P388. However, a more surprising finding was that the pattern of drug sensitivity was very similar to that for i.v. inoculated LLAK. There was a high degree of linear correlation ($r = 0.80$; $P < 0.001$) between the ILS values for the 2 tumours, and the logarithmic IC_{50} ratio was no longer a significant variable.

$$\text{ILS(LLAK)} = 1.1(\pm 0.2)\text{ILS(P388i.v.)} + 4.7$$

The lack of a significant *in vitro* term does not prove that differences in intrinsic drug sensitivity are unimportant since the active compounds cover only a narrow range of IC_{50} ratios (1–3.1). Nevertheless, the dramatic change in P388 sensitivity with alteration of tumour site suggests that factors other than intrinsic drug sensitivity are largely responsible for the apparent resistance of LLAK *in vivo*.

It is noteworthy that all amsacrine analogues with significant activity against both i.v. inoculated P388 and LLAK have IC_{50} ratios of less than 3.1. Thus, drug features which reduce the resistance of LLTC cells relative to P388 cells in culture appear to be consistent with efficient distribution to remote tumour sites *in vivo*.

In vivo *growth characteristics of the LLTC line*

Interpretation of the above results rests on the assumption that the *in vitro* cell line originally derived from LL can be considered representative of LLAK in its drug sensitivity. It was therefore of interest to determine whether the LLTC line would grow in mice, and if so whether the drug response characteristics of the resulting tumours were similar.

Subcutaneous inoculation of C57BL/J mice with 5×10^5 cultured LLTC cells resulted in the appearance of tumours at the injection site in 100% of mice. These tumours were passaged every 2 weeks, as with the LLAK line, and although growing more slowly than LLAK initially, grew at a similar rate after passaging for 8 weeks and formed lung metastases from advanced tumours. When tumours from the latter animals were disaggregated by the standard method and injected (10^6 cells) i.v. into recipients, LLTC formed lung tumours which killed all animals. The mean survival time varied from 18 to 21 days in 7 different experiments with an average of 19.9 days, and an average coefficient of variation in each experiment of 16%. This compared with 16.7 days and 15% for LLAK [2]. Examination of lungs 9 days after inoculation of 10^6 LLTC cells showed a greater heterogeneity of lung colony number, as compared with LLAK, although all lungs contained colonies.

Life extension assays were determined with a number of drugs (Table 3) for LLTC following i.v. injection of 10^6 cells. LLTC tumours were considerably more resistant to drug treatment than were LLAK tumours, both for standard agents 5-fluorouracil and cyclophosphamide, and for a range of amsacrine derivatives. However, the sensitivities

of LLTC and LLAK were significantly correlated ($r = 0.84$ $P < 0.001$).

$$ILS(LLTC) = 0.40(\pm 0.07)ILS(LLAK) - 0.9$$

Preliminary studies indicate that the resistance of LLTC relative to LLAK is related to altered cytokinetics (a lower growth fraction) in the former tumour [12].

DISCUSSION

This paper reports the results of testing of a number of clinical antitumour agents, together with a series of derivatives of the antileukaemia agent amsacrine, against 2 tumours widely used for screening for potential antitumour agents. Whereas all compounds studied are active against the i.p. implanted P388 leukaemia, only a subset (9/20) is active against i.v. inoculated LL. A P388 leukaemia cell line derived directly from the *in vivo* tumour line, and a cell line (LLTC) which was originally derived from LL [9] were used to gather *in vitro* data. Because of the different components in the growth media for the 2 cell lines, the medium for P388 cells was modified by addition of sodium ascorbate to prevent the high degradation rate of some amsacrine derivatives in RPMI medium.

Large differences in the relative sensitivities of the two cell lines to amsacrine analogues are observed in culture (Table 1), as previously noted with other cell lines [5, 6]. The use of IC_{50} to quantitate drug sensitivity depends on growth inhibition during continuous drug exposure rather than the more relevant loss of clonogenicity during short-term drug treatment. However, there are good reasons to assume that the IC_{50} values measured in this study parallel the sensitivity measured by clonogenic cell killing.

Firstly, for V79 cells in Alpha MEM we have observed (data not shown) a good correlation ($r = 0.94$; $P < 0.001$) between the IC_{50} for 45 hr drug exposure and D_{10} (drug concentration required to obtain 90% cell kill for 1 hr exposure, using compounds 3, 5, 6, 10, 12, 15, 17 and 20:

$$\log D_{10} = 0.90(\pm 0.15)\log IC_{50} + 1.38$$

Secondly, the potency of compound 20 (CI-921) against log-phase LLTC in clonogenic assays using 1 hr exposure is 2.5 times greater than amsacrine (D_{10} = 2.2±0.3 μM vs. 5.4±0.3 μM), in good agreement with the 2.1-fold higher potency of CI-921 in the growth inhibition assay (Table 1).

Reasons for the variation in relative sensitivity to the amsacrine analogues are not clear, and could reflect cell line-dependent differences in drug uptake, metabolism, interaction with targets, or sensitivity to target lesions. It is interesting to note that those analogues for which ascorbate induced the largest increase in drug activity in P388 cultures were those for which the relative resistance of LLTC was most pronounced. One possible interpretation of this observation is that LLTC cells are particularly resistant to the most readily oxidised (i.e. ascorbate-sensitive) analogues because these cells contain high levels of oxidative enzyme systems [13] capable of inactivating such drugs. However, this is not general for a larger series of anilino-acridine derivatives (unpublished results). Another possible explanation for the differences in relative sensitivity is that the cell lines vary in the efficiency of their drug efflux mechanisms. Those compounds in Table 1 which have high IC_{50} ratios also show a high level of cross-resistance (B.C. Baguley, unpublished results) in an adriamycin-resistant P388 cell line [14] which is thought to have an enhanced ability to pump out of the cell a number of antibiotics and DNA binding drugs including amsacrine [15].

Those drugs with the most favourable *in vitro* activity against LLTC cells relative to P388 cells also provided high activity against i.v. LLAK relative to i.p. P388 *in vivo* (Fig. 1). The statistically significant contribution of this *in vitro* selectivity to *in vivo* selectivity suggests intrinsic cellular sensitivity to be a major determinant of the *in vivo* tumour spectrum of these drugs. However, studies with i.v. inoculated P388 cells demonstrated this apparent correlation to be highly dependent on tumour site. Growth of P388 leukaemia cells in sites remote from the site of drug administration was sufficient to change the drug sensitivity of this tumour in such a way that it now resembled LLAK. We conclude that problems of drug access to remote tumour sites dominate the apparent difference in sensitivity between i.p. P388 and i.v. LLAK. A contribution of cell line selectivity to the *in vivo* selectivity may still be present, but this possibility is not adequately tested by the available data since those compounds with significant activity against i.v. P388 all have very similar IC_{50} ratios.

It is of considerable interest that the measured IC_{50} ratios show a correlation with *in vivo* activity against either tumour at remote sites. Thus, even though cell line selectivity may not be of primary importance *in vivo*, IC_{50} ratios may be empirically useful in identifying amsacrine analogues with high activity against remotely implanted tumours. We have recently used this approach successfully in identifying a group of anilino ring-substituted amsacrine analogues with high activity against LL tumours [16].

A problem in relating *in vivo* to *in vitro* data is that the *in vitro* LL line (LLTC) has not been recently derived from the *in vivo* LL and therefore may have diverged significantly in terms of drug sensitivity. To overcome this problem, LLTC cells were inoculated into mice and the resulting tumours were passaged to produce tumours which were quite

similar to the original Lewis lung tumour. Tumours grew in the lung following i.v. inoculation and the survival time of inoculated animals was only slightly longer than those inoculated with the original tumour. In the response to a representative subset of drugs (Table 3) it was apparent that the IC_{50} ratio could still be used to predict the active compounds, and that the increases in life-span induced in this tumour were related with a high degree of correlation to those obtained with the original tumour.

In summary, the *in vitro* sensitivity of P388 and LLTC lines show significant differences among a congeneric series of amsacrine analogues. The apparent correlation of this *in vitro* selectivity with *in vivo* response of i.p. P388 and i.v. LLTC or LLAK is an artifact imposed by the different tumour sites used in these 2 standard screening systems. These results emphasise the importance of tumour site and drug administration route in influencing apparent tumour selectivity in experimental cancer chemotherapy. For the amsacrine series, the relative sensitivity of LLTC and P388 lines appears to be empirically useful in identifying analogues with pharmacokinetic properties favouring efficient distribution *in vivo*.

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