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Doxorubicin sensitivity pattern in a panel of small-cell lung-cancer cell lines: correlation to etoposide and vincristine sensitivity and inverse correlation to carmustine sensitivity*

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Summary. The aim of our investigations is to evaluate whether the sensitivity patterns of small-cell lung-cancer (SCLC) cell lines in vitro can be used in evaluating new drugs and in selecting drugs for the optimization of combination therapy. In our attempts to obtain a panel of cell lines demonstrating differential patterns in sensitivity, we have developed three SCLC lines exhibiting different types of multidrug resistance (MDR). In the present investigations we compared the sensitivity patterns shown by five wild-type SCLC lines and three MDR lines in response to six different types of drugs: doxorubicin, cytarabine, carmustine, cisplatin, vincristine, and etoposide. In the wild-type SCLC cell lines, the range of variation in sensitivity to all drugs was within a factor of 10. Cell lines showing low sensitivity to doxorubicin also exhibited low sensitivity to etoposide and vincristine, and vice versa. In contrast, the pattern of sensitivity to carmustine was almost the opposite of that to doxorubicin. A tendency to an inverse relationship between doxorubicin and carmustine sensitivity was also observed when doxorubicin sensitivity was reduced in near stationary cells and in cells exposed to the metabolic inhibitor 2-deoxy-D-glucose. In agreement with the pattern observed for the wild-type lines, all of the MDR sublines demonstrated collateral sensitivity to carmustine. As to cytarabine, the wild-type lines expressed a sensitivity pattern similar to that shown in response to doxorubicin. Interestingly, the opposite pattern was found in the MDR lines, as all three demonstrated cytarabine hypersensitivity. The combination of alkylating agents and "MDR" drugs are of proven clinical benefit in the treatment of solid tumors, as is the combination of anthracycline and cytarabine in acute myeloid leukemia. The experimentally derived sensitivity data on cytarabine, alkylating agents, and MDR drugs (i.e., etoposide, doxorubicin, vincristine) thus resemble the clinical experience with these drugs, and we conclude that the use of a clonogenic assay on the described panel of SCLC cell lines can give valuable information for the selection of agents for combination therapy.

Introduction

Small-cell lung cancer (SCLC) is one of the solid tumors most responsive to cytostatic drugs. More than ten established anticancer agents are active against SCLC, and first-line standard treatment generally consists of combinations of some of these drugs. Although the agents are effective in the initial treatment of SCLC, most of the patients relapse with a drug-resistant tumor. As an adjuct to other preclinical drug-evaluation systems, we have been working for some years with a panel of cell lines established from patients with SCLC.

The purpose of our investigations has been to evaluate the extent to which in vitro sensitivity testing can contribute to the selection and combination of cytotoxic drugs into regimens with improved efficacy. We have shown that different drug types elicit different patterns of sensitivity [27] and that chemically closely related antineoplastic analogues with similar mechanisms of action, such as the epipodophyllotoxins etoposide and teniposide [17, 25], the platinum analogues cisplatin and carboplatin [26], the nitrosoureas tauromustine and carmustine [26], and the anthracyclines doxorubicin and daunorubicin [14] produce almost identical sensitivity profiles. Thus, it seems that the analysis of differential cytotoxicity patterns in a panel of cell lines may enable the combination of non-cross-resistant drugs and, possibly, the elucidation of the drugs' mechanism(s) of action [1, 16, 18].

In our attempts to obtain a panel of SCLC cell lines demonstrating differential patterns of sensitivity, we have developed three SCLC lines exhibiting different types of multidrug resistance (MDR). These different experimentally induced MDR cell lines include (1) a classic MDR cell line expressing P-glycoprotein (NCI-H69/DAU4)

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Table 1. DNA content, plating efficiency, relation to chemotherapy, and growth behavior in vitro of the cell lines tested

Cell line	DNA index	%PE	Prior therapy	Growth behavior
NCI-H69	0.90	12	CTX, MTX, CCNU, VCR, DOX, PRO[a]	S
NCI-H69/DAU4	0.87	12		S
NCI-H69/VP	0.82	13		S
OC-NYH	1.39	27	None	Mon
OC-NYH/VM	1.29	30		Mon
NCI-N592	1.48	30	MTX[a]	S
OC-TOL	1.40	22	None	S
GLC-16	1.80	17	CTX, VP-16, DOX	S

CTX, cyclophosphamide; MTX, methotrexate; VCR, vincristine; DOX, doxorubicin; PRO, procarbazine; PE, plating efficiency at approximately 3000 colonies; S, growth in suspension; Mon, growth as a monolayer; ND, not determined

[a] D. Carney, personal communication

[10], (2) an MDR subline that is cross-resistant to vincristine but apparently does not express P-glycoprotein (NCI-H69/ VP), and (3) an MDR cell line whose resistance is due to altered topoisomerase II activity (at-MDR; OC-NYH/VM [6]. In the present investigations, we compared the sensitivity patterns shown by five wild-type SCLC lines and the three MDR lines in response to six different types of drugs: doxorubicin, cytarabine, carmustine, cisplatin, vincristine, and etoposide.

Materials and methods

Drugs. Doxorubicin (Adriamycin; Carlo Erba), cytarabine (Ara-C, cytosine arabinoside; Upjohn), and vincristine (Oncovin; Lilly) were dissolved in sterile water just prior to their use. Carmustine (BCNU; Bristol-Myers Squibb) was dissolved in 10% (v/v) ethanol in sterile water. Etoposide (VP-16; Bristol-Myers Squibb) and cisplatin (Bristol-Myers Squibb) were obtained in solution for infusion at 20 and 0.5 mg/ml, respectively. All drugs were diluted more than 100 times with tissue-culture medium just before their use.

Cell lines. The human SCLC cell lines used were NCI-H69 [4], NCI-N592 [4], OC-NYH [21], OC-TOL [21], and GLC-16 [3]. Cell line NCI-H69/DAU4 was maintained in 0.1 μg daunorubicin/ml [14]. The partial revertant NCI-H69/DAU8 [14] was maintained in drug-free medium for 8 weeks before testing. Resistance to etoposide and teniposide was obtained by treating NCI-H69 and OC-NYH cells continuously with increasing concentrations of the respective drugs. After 6 months, the NCI-H69/VP cells grew in 3 μM etoposide (VP-16), and the subline was maintained for 3 months at this concentration. After 6 months, OC-NYH/VM cells grew in 0.25 μM teniposide (VM-26), and the subline was maintained for 3 months at this concentration [18]. Resistant cell lines were grown in vitro in the absence of drug for 5 days before testing.

All cell lines were maintained at 37° C in RPMI 1640 supplemented with 10% fetal calf serum in a humidified atmosphere containing 7.5% CO_2. The cell lines were free of mycoplasm contamination, and the stability of their DNA content was checked by flow-cytometric DNA analysis [33]. The DNA content, plating efficiency, relation to chemotherapy, and growth behavior in vitro of the cell lines used are described in Table 1.

Clonogenic assay. Drug toxicity was assessed by colony formation in soft agar on a feeder layer containing sheep red blood cells as previously described [24]. Single-cell suspensions ($1-4 \times 10^4$ cells/ml) in RPMI 1640 supplemented with 10% fetal calf serum were exposed to the drugs for 72 h. Cells were then vortexed and pelleted, washed twice with phosphate-buffered saline (PBS) at 20° C, and disaggregated with 10 strokes of a 1-ml syringe equipped with an 18-gauge needle. The numbers of cells plated were adjusted to obtain 2,000–3,000 colonies in the control dishes. The impact on sensitivity of varying the preexperimental growth conditions was studied in cell line NCI-N592 using 1-h periods of drug exposure [15]. Clonogenic assay was performed on cells harvested from cultures in which 5×10^5 cells in 15 ml medium had been incubated 2 and 8 days earlier [15]. In other experiments, NCI-N592 cells were incubated with 10 mM 2-deoxy-D-glucose for 24 h before drug exposure to reduce the sensitivity to etoposide [13]. In each experiment the drugs were tested on the same batch of cells to reduce the interexperimental variation [15]. In experiments involving continuous drug exposure, the cells were plated directly in agar with the desired drug concentration. The colonies were counted after a 3-week incubation period.

Data analysis. The cells were exposed to five concentrations of each drug. All experiments were done in triplicate. To obtain linearity on exponential dose-response curves, the logarithmically transformed response data were used in linear regression analysis. The dose reducing the number of colonies to 50% of the control value (LD_{50}) was computed from the regression parameters [15].

Detection of P-glycoprotein by photoaffinity labeling with azidopine and by Western-blot analysis. For photoaffinity labeling, 1×10^6 cells in 50 μl PBS were incubated with 0.5 μM [^{3}H]-azidopine and UV-irradiated at 254 nm for 10 min as described by Safa et al. [28]. The cells were lysed with 2% NP40 and analyzed on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel as previously described [30]. For Western-blot analysis, $1-5 \times 10^7$ cells were washed with PBS and resuspended (2 : 3, v/v) in 1% NP40 as described elsewhere [14]. After centrifugation at 1,400 *g*, equal amounts of protein (Bradford protein assay Bio-Rad, Calif., USA) from the supernatants was loaded onto a 10% SDS-PAGE gel. Proteins were blotted onto nitrocellulose paper after electrophoresis and probed with monoclonal antibodies C219 (Centocor, Pa., USA) or JSB-1 (Sanbio, Holland) against P-glycoprotein [20, 29].

Results

Sensitivity pattern in five wild-type SCLC cell lines

Figure 1 shows the LD_{50} values obtained in five wild-type SCLC cell lines tested with doxorubicin, cytarabine, carmustine, cisplatin, vincristine, and etoposide. The cell lines are sorted according to increasing sensitivity to doxorubicin. The difference between doxorubicin-sensitive and less sensitive lines lay within a factor of only 3. Patterns of sensitivity to vincristine and etoposide were almost identical and were very similar to that produced by doxorubicin. Remarkably, the pattern of sensitivity to carmustine was almost the opposite of that to doxorubicin. Thus, the two lines most resistant to carmustine were those that were most sensitive to doxorubicin. This pattern is in agreement with the results we previously obtained using carmustine [26], etoposide [25], and cisplatin [27] in the four wild-type lines NCI-H69, NCI-N592, OC-NYH, and OC-TOL. Thus, in these experiments involving 1-h drug exposure, cell lines NCI-H69 and NCI-N592 were most resistant to etoposide and more sensitive to carmustine and cisplatin than were cell lines OC-NYH and OC-TOL.

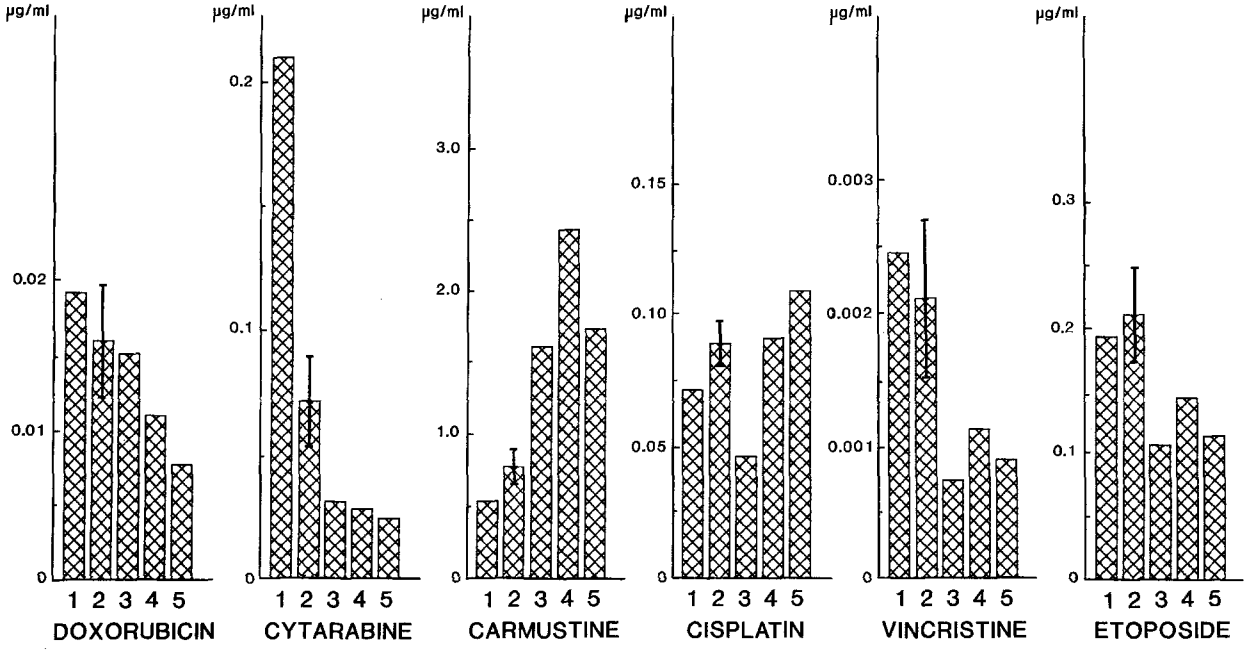

Fig. 1. Sensitivity patterns of five wild-type SCLC cell lines. The cells were exposed to drugs for 72 h prior to plating. The cell lines are sorted according to increasing sensitivity to doxorubicin. The drug concentration (µg/ml) reducing the number of surviving colonies to 50% of control values (LD_{50}) are shown. *1*, NCI-N592; *2*, NCI-H69; *3* GLC-16; *4*, OC-TOL; *5*, OC-NYH. The bars in *2* represent standard deviations from 3 independent experiments

Table 2. Mean LD_{50} values determined for NCI-N592 cells tested on days 2 and 8 after passage[a]

	DOX	BCNU	CISPT	VP-16
Day 2	0.18 (0.04)	1.9 (0.5)	3.1 (1.6)	11 (3)
Day 8	0.47 (0.09)	1.3 (0.1)	2.3 (0.4)	14 (1)

[a] Cells were exposed to drug for 1 h. Data represent mean values (in µg/ml) for 3 separate experiments; standard deviations are given in parentheses

Preexperimental growth conditions and sensitivity pattern

Subsequently, the impact of varying cell-growth conditions on the sensitivity pattern was studied. NCI-N592 cells harvested at 2 or 8 days after passage were exposed to drugs for 1 h and plated; the results are shown in Table 2. We have previously demonstrated that the fraction of NCI-N592 cells in the S phase and the sensitivity to doxorubicin are higher in cells harvested early after passage (day 3) than in those harvested later (day 7) [15]. This variation in doxorubicin sensitivity was also observed in the present study. Although it did not reach statistically significance, a similar trend was seen in the sensitivity of cells to etoposide, and the variation in sensitivity to cisplatin and carmustine was again complementary to that found for doxorubicin, as these drugs were most efficient in cells tested on day 8 (Table 2).

To study further this inverse relationship, we evaluated the effect of 2-deoxy-D-glucose on drug cytotoxicity. This metabolic inhibitor has been shown to reduce cellular sensitivity to etoposide [13]. The effect of 10 mM 2-deoxy-D-glucose preincubation for 24 h on the sensitivity to doxorubicin, carmustine, cisplatin, and etoposide of cell line NCI-N592 was compared with nonexposed controls. Apparently 2-deoxy-D-glucose had no influence on plating efficiency or on cell viability. As seen in Table 3, the sensitivity to etoposide and doxorubicin was reduced and the sensitivity to carmustine and cisplatin was increased by treating the cells with 2-deoxy-D-glucose. Thus, it seems that the complementary sensitivity pattern can be obtained merely by varying cell-growth conditions prior to sensitivity testing.

Table 3. LD_{50} values determined for NCI-N592 cells tested on day 5 after passage in the presence or absence of 24 h preincubation with 10 mM 2-deoxy-D-glucose[a]

	DOX Experiment		BCNU Experiment		CISPT Experiment		VP-16 Experiment		Dead % Experiment		PE % Experiment	
	1	2	1	2	1	2	1	2	1	2	1	2
Day 5	0.15	0.13	3.3	2.1	5.5	3.0	8	6	41	35	40	20
Day 5 + 2dglu	0.24	0.20	2.7	1.5	2.4	1.9	18	11	33	30	30	15

[a] Cells were exposed to drug for 1 h; LD_{50} (µg/ml) values were obtained in 2 separate experiments. Dead %, Percentage of cells not excluding nigrosin dye; PE%, plating efficiency (percentage of plated viable cells that give rise to colonies); DOX, doxorubicin; BCNU, carmustine; VP-16, etoposide; 2dglu, 2-deoxy-D-glucose

Table 4. Mean LD_{50} values obtained in 3 and 4 independent experiments following 72 h drug exposure in NCI-H69 and NCI-H69/DAU4 cell lines, respectively

Drug	NCI-H69 ($n = 3$; μg/ml)		NCI-H69/DAU4 ($n = 4$; μg/ml)		RR
	Mean	SD	Mean	SD	
DOX	0.016	0.004	0.078	0.007	4.4*
ARA-C	0.070	0.018	0.031	0.011	0.4
BCNU	0.77	0.13	0.34	0.06	0.4*
CISPT	0.089	0.009	0.033	0.007	0.4*
VCR	0.0021	0.0006	0.0116	0.0037	5.5*
VP-16	0.21	0.04	0.55	0.10	2.6*

DOX, doxorubicin; ARA-C, cytarabine; BCNU, carmustine; CISPT, cisplatin; VCR, vincristine; VP-16, etoposide; RR, relative resistance of NCI-H69/DAU4

* Significant difference in sensitivity between the two lines

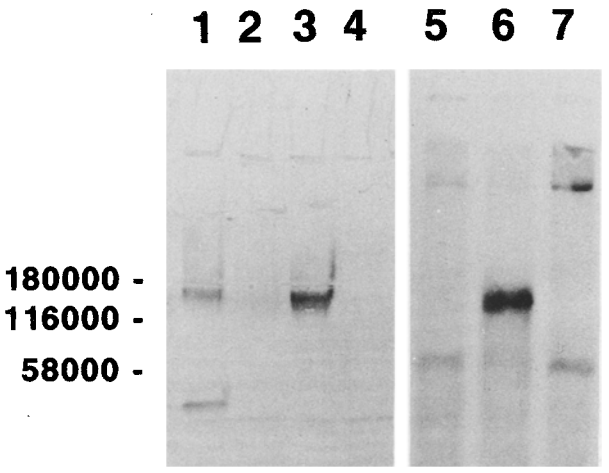

Fig. 2. Western-blot detection and photoaffinity labeling of P-glycoprotein. In lanes *1–4*, P-glycoprotein was detected with C219 monoclonal antibody. *Lane 1*, Ehrlich/DNR-positive control; *lane 2*, NCI-H69/VP; *lane 3*, H69/DAU4; *lane 4*, OC-NYH/VM. *Numbers to the left* represent molecular-weight markers (Da). In *lanes 5–7*, the cells were labeled with 0.5 μM [^{3}H]-azidopine. Fluorographs were obtained after SDS-PAGE. *Lane 5*, NCI-H69/VP; *lane 6*, NCI-H69/DAU4; *lane 7*, NCI-H69

Sensitivity patterns in MDR SCLC lines

The six drugs were also tested on a classic MDR cell line that was resistant to vincristine and expressed P-glycoprotein (NCI-H69/DAU4; Table 4), on an MDR subline that was cross-resistant to vincristine but apparently did not express P-glycoprotein (NCI-H69/VP) (Fig. 2), and on an MDR cell line (OC-NYH/VM) whose resistance was due to altered topoisomerase II activity [18]. The resultant LD_{50} values are depicted in Fig. 3. Both the results obtained in the parental wild-type lines NCI-H69 on OC-NYH and those obtained in a partial revertant of NCI-H69/DAU4 called NCI-H69/DAU8 (line 3 in Fig. 3) that was without detectable P-glycoprotein [14] are included for comparison.

All three sublines of NCI-H69 [NCI-H69/VP (line 1 in Fig. 3), NCI-H69/DAU4 (line 2 in Fig. 3), and NCI-H69/DAU8 (line 3 in Fig. 3)] were cross-resistant to doxorubicin, vincristine, and etoposide. The NCI-H69/VP subline made resistant to etoposide contained no detectable

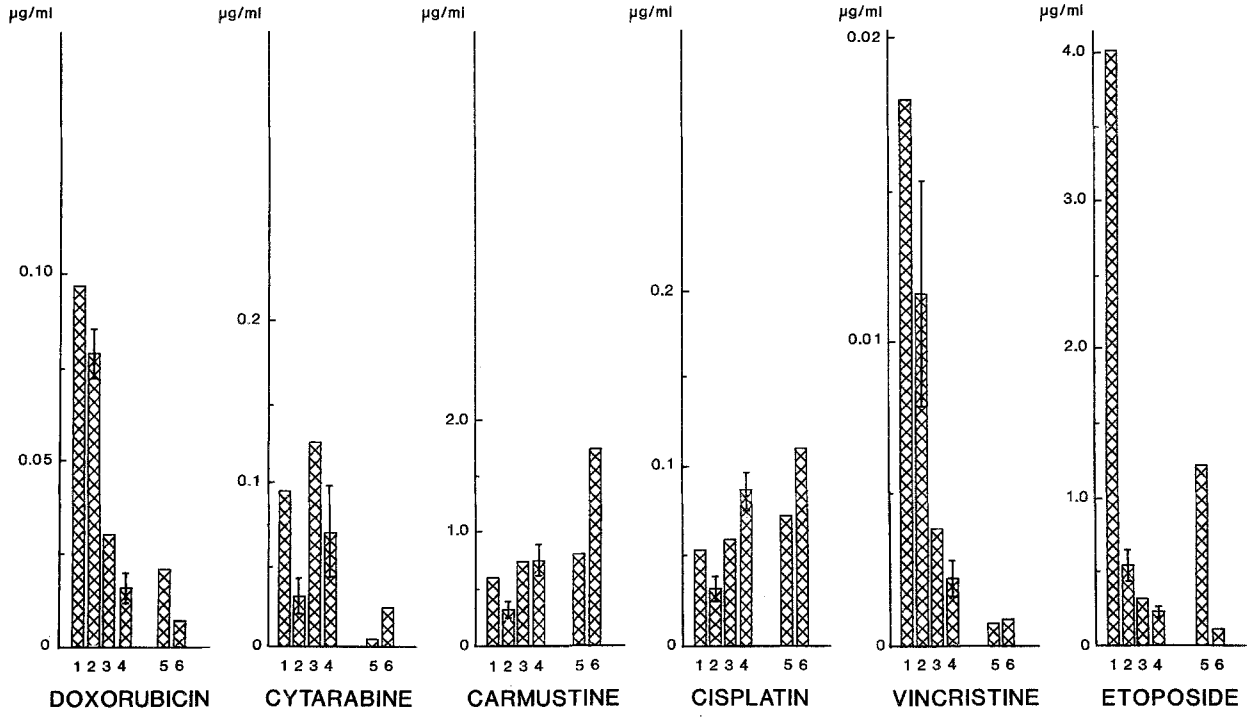

Fig. 3. Sensitivity patterns of four experimentally induced MDR lines and their parental wild-type SCLC cell lines. The cells were exposed to drugs for 72 h prior to plating. The cell lines are sorted according to increasing sensitivity to doxorubicin. The drug concentration (μg/ml) reducing the number of surviving colonies to 50% of control values (LD_{50}) are shown. *1*, NCI-H69/VP; *2*, NCI-H69/DAU4; *3*, NCI-H69/DAU8; *4*, NCI-H69; *5*, OC-NYH/VM; *6*, OC-NYH. The bars in *2* and *4* represent standard deviations from at least 3 independent experiments

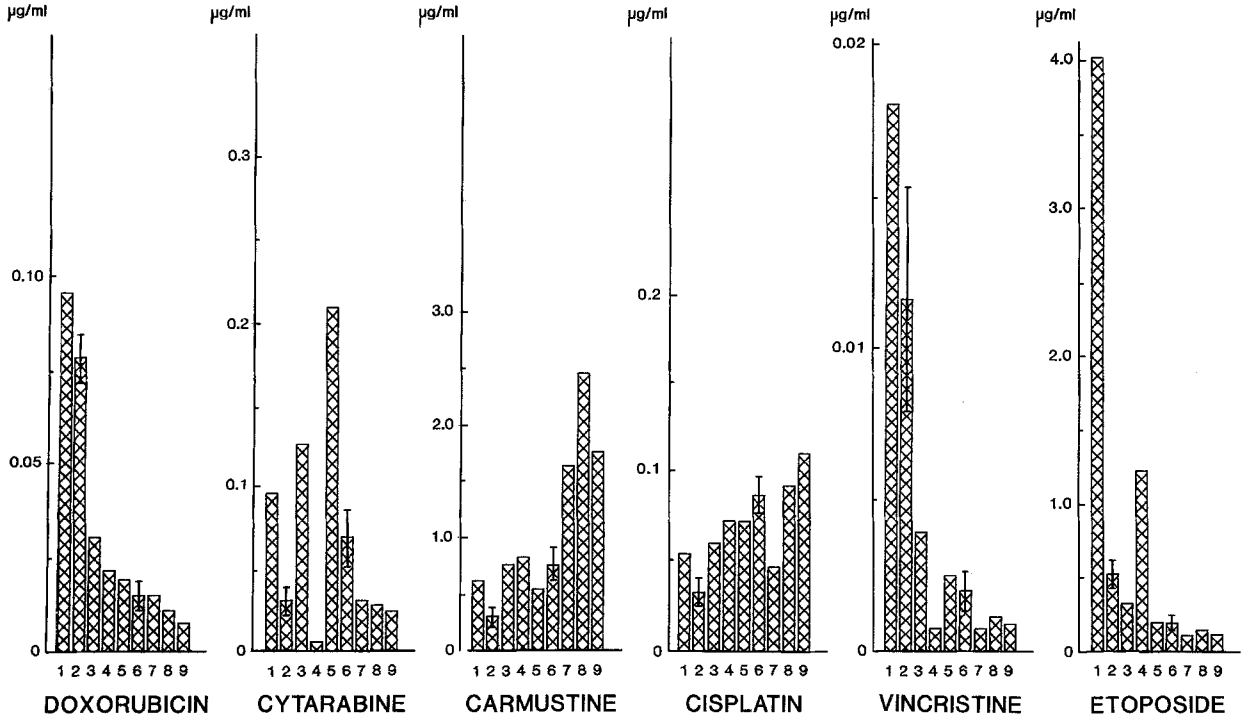

Fig. 4. Merged sensitivity patterns from Figs. 1 and 3. The cell lines are sorted according to increasing sensitivity to doxorubicin. The drug concentration (µg/ml) reducing the number of surviving colonies to 50% of control values (LD_{50}) are shown. *1*, NCI-H69/VP; *2*, NCI-H69/DAU4; *3*, NCI-H69/DAU8; *4*, OC-NYH/VM; *5*, NCI-N592; *6*, NCI-H69; *7*, GLC-16; *8*, OC-TOL; *9*, OC-NYH. Bars represent standard deviations from at least 3 independent experiments

Table 5. Relative resistance determined for the MDR lines NCI-H69/DAU4, NCI-H69/VP, and OC-NYH/VM as compared with the wild-type parent lines NCI-H69 and OC-NYH, respectively, in a clonogenic assay using continuous drug exposure[a]

	DOX	ARA-C	BCNU	CISPT	VCR	VP-16
NCI-69/DAU4	3	0.3	0.4	0.7	>5	3
NCI-69/VP	4	0.6	0.5	0.6	4	>3
OC-NYH/VM	8	0.6	0.7	1	1	18

[a] Data represent the ratio of the LD_{50} value found for the resistant subline over that determined for the wild-type parent line. The LD_{50} values used in the computations represent the mean of 3 determinations in the wild-type lines and the mean of 2 determinations in the resistant lines

DOX, doxorubicin; ARA-C, cytarabine; BCNU, carmustine; CISPT, cisplatin; VCR, vincristine; VP-16, etoposide

P-glycoprotein but was nevertheless more resistant to doxorubicin than was the P-glycoprotein-expressing NCI-H69/DAU4 subline (Fig. 2). All three sublines of NCI-H69 were more sensitive to carmustine and cisplatin than was the parental cell line NCI-H69 (line 4 in Fig. 3). The OC-NYH/VM (line 5 in Fig. 3) subline of OC-NYH (line 6) made resistant to teniposide was cross-resistant to etoposide and doxorubicin but not to vincristine; moreover, it showed collateral sensitivity to carmustine and cisplatin. Cytarabine was the only drug whose sensitivity pattern changed, exhibiting cross-resistance to doxorubicin in the wild-type lines and collateral sensitivity to doxorubicin in all three MDR lines.

Finally, the three MDR sublines were compared with their parental wild-type lines in experiments involving continuous drug exposure. The use of continuous drug exposure has the advantage of being technically simpler, as the cells are plated directly in agar with the desired drug concentrations. In these experiments, all three MDR lines exhibited resistance to doxorubicin and etoposide as well as collateral sensitivity to carmustine and cytarabine, and NCI-H69/DAU4 and NCI-H69/VP exhibited vincristine resistance and enhanced cisplatin sensitivity (Table 5). We observed a lack of cross-resistance but no collateral sensitivity to cisplatin in OC-NYH/VM cells. Thus, as compared with the results obtained using 72-h exposure periods, continuous drug exposure reproduced 17 of 18 findings on the pattern of collateral sensitivity and cross-resistance in the three resistant cell lines (Tables 4, 5). Figure 4 depicts all data from Figs. 1 and 3. The cell lines are sorted according to increasing sensitivity to doxorubicin, and this figure demonstrates the inverse relationship between the sensitivity to MDR drugs and the sensitivity to alkylating agents.

Discussion

The aim of the present investigations was to evaluate whether the sensitivity patterns of SCLC cell lines in vitro could be used in disease-specific evaluation of new drugs and in selecting drugs for the optimization of combination therapy.

In the selected panel of wild-type lines, it has previously been shown that drug analogues (e.g., etoposide and teniposide) with similar mechanisms of action produce identical sensitivity patterns [17, 25]. Interestingly, the

patterns of sensitivity to etoposide and vincristine observed in the five wild-type lines were analogous and very similar to those produced by doxorubicin. Thus, drugs with different mechanisms of action may demonstrate identical sensitivity patterns. This result could indicate that the mechanisms of MDR found in experimentally resistant cell lines are also relevant in determining the patterns of sensitivity of wild-type cell lines. This notion agrees with the correlation between etoposide and doxorubicin sensitivity demonstrated by Carmichael et al. [5] using a tetrazolium-dye (MTT) sensitivity assay in 15 wild-type SCLC cell lines. In addition, Carmichael et al. [5] found a significant correlation between cisplatin and carmustine sensitivity, whereas no correlation was observed between etoposide and cisplatin sensitivity.

Two well-defined mechanisms of MDR have been identified:

1. Active extrusion of the toxic compound involving a protein (P-glycoprotein) in the plasma membrane reduces the drug concentration at the target (classic MDR) [10].

2. Altered (reduced) topoisomerase II activity decreases the target's sensitivity to anthracyclines and epipodophyllotoxins (at-MDR) [6].

We were unable to detect P-glycoprotein in our panel of wild-type cell lines using Western-blot analysis [14]. In the resistant sublines, we detected the protein using monoclonal antibody C219 in NCI-H69/DAU4 but not in the partial revertant subline NCI-H69/DAU8 [14] or in NCI-H69/VP (Fig. 2). Positive staining in NCI-H69/DAU4 was also obtained using antibody JSB-1, whereas NCI-H69/VP was negative (data not shown). Likewise, photoaffinity labeling with azidopine [28] enabled the detection of P-glycoprotein in NCI-H69/DAU4 but not in NCI-H69/VP (Fig. 2). Thus, P-glycoprotein expression apparently explains the MDR pattern of NCI-H69/DAU4 only. The cell line OC-NYH/VM fulfills the criteria for at-MDR, exhibiting reduced topisomerase II activity and cross-resistance to etoposide and doxorubicin but not to vincristine [18]. OC-NYH/VM was the only cell line in which no cross-resistance between etoposide and vincristine was observed.

In the wild-type lines, the correlation between etoposide and vincristine sensitivity excluded alterations in topoisomerase II as the sole explanation for the sensitivity variation. This finding is corroborated by the results obtained using NCI-H69/VP, which was made resistant to the selective topoisomerase II targeting etoposide but also showed cross-resistance to vincristine. Thus, the MDR pattern detected in NCI-H69/VP and, possibly in the wild-type lines as well does not fit into either the classic Pgp-MDR or at-MDR definition. In this context, it should be noted that we do not know the sensitivity of our P-glycoprotein detection and therefore cannot exclude the possibility that there is a low overexpression of P-glycoprotein in the NCI-H69/VP subline as compared with the wild-type NCI-H69 line. However, the apparently P-glycoprotein-negative NCI-H69/VP line was more resistant to doxorubicin than was the P-glycoprotein-positive NCI-H69/DAU 4 line, and additional mechanisms of resistance thus seem to be active in NCI-H69/VP.

Drug combinations in which cells that are resistant to one drug show enhanced sensitivity to another agent would be of obvious clinical value. Thus, a possible explanation for the clinical synergy seen between epipodophyllotoxin and cisplatin against SCLC [23] could involve the increased activity of platinum analogues against subpopulations exhibiting decreased sensitivity to epipodophyllotoxins [11]. As early as 1971, Danø [7, 8] reported observations of collateral sensitivity to carmustine and cytarabine in the first papers on the development of resistance to daunorubicin and doxorubicin in Ehrlich ascites cells. Similarily, P388 leukemia made resistant to 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide (m-AMSA) was collaterally sensitive to alkylating agents [19]. Collateral sensitivity to doxorubicin was found in two melanoma cell lines selected for resistance to alkylating agents [34] and in four lung-cancer cell lines rendered resistant to cisplatin [22].

In the present study, increased sensitivity to carmustine was obtained whenever cells showed decreased sensitivity to etoposide and doxorubicin. The decreased etoposide sensitivity may be due either to changes in cell growth (Table 2) or metabolism (Table 3) or to stable resistance. These findings are in agreement with the results of Barcellos-Hoff et al. [2], who found that noncycling 9L cells reentering the cell cycle were more susceptible to carmustine than were log-phase cycling 9L cells. Topoisomerase II activity is low in noncycling cells [12], and it has been suggested that topoisomerases are necessary for DNA repair [31]. Thus, it is tempting to attribute the inverse relationship to variations in topoisomerase activity. A low topoisomerase II level would result in low sensitivity to doxorubicin and etoposide and in high sensitivity to carmustine, and vice versa. In this context, it is noteworthy that Tan et al. [32] found that cells made resistant to nitrogen mustard exhibited elevated topoisomerase II activity and increased sensitivity to etoposide.

The mechanisms underlying the variations in sensitivity are, of course, important, especially if one seeks to modulate clinical resistance, e.g. with verapamil. Even in the most successful experiments using verapamil and other modulators of classic MDR, it is seldom possible to restore sensitivity to wild-type levels. The present investigations suggest that is is more effective to combine MDR drugs with alkylating agents or to use alkylating agents or cytarabine in resistant lines than to attempt to modulate the resistance to MDR drugs. It is possible that the scheduling of MDR drugs prior to the use of alkylating agents is advantageous in tumors in which the MDR phenotype is not consistently expressed at diagnosis but is detected at the time of first relapse [9]. This idea is supported by the collateral sensitivity to cytarabine and carmustine observed in MDR cells.

In conclusion, the experimentally derived data on sensitivity to alkylating agents, cytarabine, and MDR drugs correspond to the clinical experience gained with these drugs. Thus, the use of a clonogenic assay on the described panel of SCLC cell lines can give valuable information for the selection of agents for combination therapy.

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