Differential Activity of Cremophor EL and Paclitaxel in Patients' Tumor Cells and Human Carcinoma Cell Lines In Vitro

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BACKGROUND. Previous studies indicate that Cremophor EL (CEL), the excipient for Taxol, a clinical preparation of paclitaxel, has biologic properties per se.

METHODS. The cytotoxic activity of Taxol and its solvents CEL/ethanol, paclitaxel in ethanol, and 14 other cytotoxic drugs was investigated in vitro in 10 human carcinoma cell lines and 183 tumor samples from patients with tumors of various types. Cytotoxicity was determined by the fluorometric microculture cytotoxicity assay.

RESULTS. In the cell lines, Taxol was generally more active than paclitaxel; this may have been due to an additive effect of the diluent. This activity was pronounced in sublines expressing tubulin-associated and P-glycoprotein-mediated drug resistance, indicating involvement of these mechanisms in paclitaxel resistance and their modulation by CEL. Taxol and paclitaxel were highly cross-resistant to other tubulin-active agents, whereas the low cytotoxic effect of CEL seemed unrelated to other drugs. In the samples from patients, Taxol was less active than in the cell lines but showed a differential activity that corresponded reasonably well with that in the clinic. CEL and Taxol were similarly active, indicating that paclitaxel did not add substantially to the activity of Taxol.

CONCLUSIONS. Whereas the cell line data clearly confirmed the well-known properties of paclitaxel, a more valid model using tumor cells from patients demonstrated that CEL significantly contributes to the efficacy of Taxol in vitro. The clinical relevance of this finding remains to be elucidated. ***Cancer* 1997;79:1225–33.**

KEYWORDS: Cremophor EL, paclitaxel, Taxol, human tumor cell, in vitro.

Multidrug resistance (MDR) may contribute to the failure of chemotherapy in human cancer and is thought to be partly mediated by the overexpression of P-glycoprotein (P-gp), a 170-kilodalton plasma membrane protein. Cremophor EL (CEL; ethoxylated castor oil) and other fatty acid ester surfactants have been found to reverse P-gp-mediated MDR and inhibit cell cycle traversing in vitro in cell lines.[1–4] Surfactants are, therefore, of potential clinical interest as anticancer agents.[5,6] The former are complex mixtures of incompletely characterized components synthesized from undefined natural products (e.g., castor oil) or from industrial grade fatty acid preparations.[4]

The molecular heterogeneity is not a problem when surfactants are used in their customary role as excipients. However, the fact that the newly introduced anticancer drug paclitaxel for clinical use is presently formulated in CEL/ethanol (Taxol; Bristol-Myers Squibb, Bromma, Sweden) has led to the intriguing hypothesis that the surfactant may contribute to its clinical activity,[1,3,5,7] especially because

Taxol has shown activity against a broad range of cancers, some of which are considered to be refractory to conventional chemotherapy.[8] The finding that CEL has profound effects on paclitaxel pharmacokinetics in vivo also clearly indicates that the excipient is not only a passive bystander.[9]

Therefore, the current study was undertaken to investigate in vitro the cytotoxic activity of CEL in the Taxol preparation. CEL was used alone and compared with various formulations of paclitaxel and standard cytotoxic drugs in tumor samples from patients with hematologic or solid tumors and in ten human carcinoma cell lines representing different resistance phenotypes.

MATERIALS AND METHODS

Cell Lines

The cell line panel included the myeloma cell line RPMI 8226 and its sublines 8226/Dox40 and 8226/LR-5 (gifts of W. S. Dalton, Department of Medicine, Cancer Center Division, University of Arizona, Tuscon, AZ), the human histiocytic lymphoma cell line U937-GTB and its subline U937-Vcr (gifts of K. Nilsson, Department of Pathology, University of Uppsala, Sweden), the small cell lung carcinoma cell line NCI-H69 and its subline H69AR (American Type Culture Collection; ATCC, Rockville, MD), the renal adenocarcinoma cell line ACHN (ATCC), and the leukemic cell line CCRF-CEM and its subline CEM/VM-1 (gifts of W. T. Beck, Dept. of Pharmacology, College of Medicine, University of Tenessee, Memphis, TN).

The 8226/Dox40 subline selected for doxorubicin (Dox) resistance shows the classic MDR phenotype with overexpression of P-gp,[10,11] whereas the melphalan resistance of the 8226/LR-5 subline is associated with an increased level of glutathione.[12,13] The vincristine (Vcr) resistance of the U937-Vcr subline is proposed to be tubulin-associated,[14] and the Dox-resistant H69AR subline expresses an MDR phenotype proposed to be mediated by the multidrug resistance-associated protein (MRP).[15,16] The teniposide-resistant subline CEM/VM-1 expresses topoisomerase II-associated atypical MDR.[17,18] The ACHN cell line is primarily drug resistant, the mechanisms of which have not yet been detailed.[19]

The cell lines were grown in culture medium RPMI 1640 (HyClone, Cramlington, United Kingdom), supplemented with 10% heat-inactivated fetal calf serum (HyClone), 2 mM glutamine 50 μg/mL streptomycin, and 60 μg/mL penicillin (HyClone), in an atmosphere containing 5% carbon dioxide. The sublines were regularly exposed to the cytotoxic drugs used for development of drug resistance,[10–18] and maintenance of drug resistance was ascertained by regular testing.

TABLE 1
IC50s (μg/mL) in the Ten Cell Lines for CEL, Paclitaxel, and Taxol, as well as Paclitaxel/Taxol Activity Ratios[a]

Cell lines	CEL IC50	Paclitaxel IC50	Taxol IC50	Paclitaxel/Taxol activity ratio
U937-Vcr	4.0	0.31	0.02	16
U937-GTB	7.0	0.01	0.01	1
8226/LR-5	1.2	0.01	0.01	1
8226/Dox40	2.0	7.00	0.65	11
8226/S	2.0	0.01	0.01	1
H69AR	0.5	0.20	0.10	2
NCI-H69	4.4	100	4.00	25
ACHN	1.0	35.0	7.00	5
CEM/VM-1	3.0	0.08	0.01	8
CCRF-CEM	3.5	0.06	0.01	6

CEL: Cremophor EL; IC50: concentration giving a survival index of 50%.

[a] Paclitaxel/Taxol activity ratio was obtained by dividing the IC50 for paclitaxel with that of Taxol. The CEL IC50s are expressed as the corresponding concentrations of Taxol. Data are presented as the mean value of two to three experiments.

Tumor Samples and Cell Preparation

A total of 183 patient samples (111 hematologic and 72 solid tumors) were obtained from patients undergoing bone marrow/peripheral blood sampling, routine surgery, or diagnostic biopsy. The sampling was approved by the research ethical committee at Uppsala University Hospital. In the case of a limited number of cells, only the drugs considered most important for that sample were tested at their empirically derived cutoff concentration (EDCC), which is the concentration of each drug giving the largest scatter of cell survival in the assay.[20,21]

Tumor cells were prepared by collagenase digestion and Percoll (Kabi-Pharmacia, Uppsala, Sweden) or Ficoll-Isopaque (Kabi-Pharmacia) density gradient centrifugation depending on the type of sample.[20,21] The proportion of tumor cells was judged by light microscopic examination of May-Grünwald-Giemsa-stained cytocentrifuged cells. Culture medium RPMI 1640 (HyClone) supplemented as described earlier was used throughout. Some cell preparations were cryopreserved in liquid nitrogen before analysis. This has been found not to alter the cytotoxic drug sensitivity.[20]

Reagents and Drugs

The CEL and paclitaxel formulations used were CEL (Sigma, St. Louis, MO) in an equal volume of ethanol, paclitaxel (Sigma) dissolved in ethanol (referred to as paclitaxel later), and the clinical formulation of paclitaxel, Taxol (Bristol-Myers Squibb), containing 6 mg/mL paclitaxel in 50% CEL and 50% ethanol (volume/volume). Control experiments found CEL to show all and ethanol none of the activity of the CEL-ethanol

TABLE 2
Correlation Coefficients between CEL (First Column) or Taxol (Second Column) and Other Cytotoxic Drug log IC50s in the Ten Cell Lines[a]

Rank order	Drug[b]	CC	Mean IC50 (μg/mL)	Drug	CC	Mean IC50 (μg/mL)
1	CEL	1.00	4.14	Taxol	1.00	1.10
2	5-Azacytidine	0.002	0.05	Paclitaxel	0.95[e]	16.0
3	Paclitaxel	−0.09	16.0	Colchicine	0.75[d]	30.0
4	Taxol	−0.11	1.10	Vincristine	0.75[d]	24.2
5	5-Fluorouracil	−0.13	63.4	Vinblastine	0.74[d]	14.6
6	6-Mercaptopurine	−0.22	26.0	Taxotere	0.71[d]	25.2
7	6-Thioguanine	−0.22	2.82	Vinorelbine	0.71[d]	12.0
8	Cisplatin	−0.36	4.53	Cisplatin	0.68[c]	4.53
9	Cytarabine	−0.37	60.0	Doxorubicin	0.56	1.90
10	Vincristine	−0.64[c]	24.2	5-Fluorouracil	0.47	63.4
11	Etoposide	−0.65[c]	22.4	4-HC	0.41	1.00
12	Doxorubicin	−0.75[d]	1.90	Etoposide	0.39	22.4
13	4-HC	−0.84[e]	1.00	Cytarabine	0.18	60.0

CC: correlation coefficients; CEL: Cremophor EL; IC50: concentration giving a survival index of 50%; 4-HC: 4-hydroperoxycyclophosphamide.

[a] The table shows the correlation coefficients (CC) between Cremophor EL-ethanol (CEL) or Taxol and other cytotoxic drugs for log concentrations giving a survival index of 50% (IC50) for the ten cell lines as well as the mean IC50s for the indicated drugs. The CEL concentration is expressed as the corresponding Taxol concentration. The 12 drugs with the highest correlations to CEL and Taxol, respectively, are included and presented in rank order according to decreasing correlation coefficients. 4-hydroperoxycyclophosphamide is the active metabolite of cyclophosphamide.

[b] Drugs and their origin/solvent: 5-azacytidine: Sigma (St. Louis, MO)/phosphate-buffered saline (PBS); colchicine: Sigma/sterile water; 6-mercaptopurine: Sigma/0.04M NaOH; Taxotere: Rhône-Poulenc Rorer (Heltinborg, Sweden)/ethanol; 6-thioguanine: Sigma/0.04M NaOH; vinblastine: Lilly (Indianapolis, IN)/PBS; Vinorelbine: Pierre Fabre Medicament (Boulogne, France)/sterile water. Data based on one typical experiment.

[c] $P < 0.05$.

[d] $P < 0.01$.

[e] $P < 0.001$.

mixture (data not shown), which will be referred to as CEL later in the text. The drugs were further diluted in sterile water, and for assessment of dose-response relationships were tested at the concentration range 0.2–125 μg/mL for paclitaxel and Taxol, and 0.03–20.8 μl/mL for CEL, which corresponds to the solvent content of Taxol in its concentration range.

The other cytotoxic drugs tested, their origin, solvents, and concentrations used for activity evaluation were as described. Experimental V-shaped 96-well microtiter plates (Nunc, Roskilde, Denmark) were prepared with 20 μL/well of drug solution at 10 times the desired final concentration.[20] The plates were stored frozen at −70 °C until use. Drug stability during storage conditions was ascertained by repeated testing of sensitive cell lines (data not shown).

Assay Procedure

The principal steps of the fluorometric microculture cytotoxicity assay (FMCA) have been described previously.[20,21] Briefly, on Day one 180 μL/well of the tumor cell preparation (5000–20,000 cells/well for the cell lines and 10,000–20,000 and 50,000–100,000 cells/well for solid and hematologic tumors, respectively) was added to microtiter plates prepared as described.[20] The plates were then incubated at standard culture conditions for 72 hours followed by washing in buffer and then addition of buffer containing 10 μg/mL fluorescein diacetate. After incubation for 30–60 minutes at 37 °C the fluorescence from each well was measured in a Fluoroscan 2 (Labsystems OY, Helsinki, Finland). Quality criteria for a technically successful assay for patient samples included a fluorescence signal in control cultures of ≥5 × mean blank values, mean coefficient of variation (CV) in control cultures of ≤ 30% and ≥ 70% tumor cells prior to incubation,[22] and for the cell lines ≥ 90% cell viability prior to incubation, a fluorescence signal in control cultures of ≥10 × mean blank values, and a CV of ≤ 30%. Only data from successful assays were included.

Quantification of FMCA Results

The FMCA results are presented as survival index (SI), defined as fluorescence of test in percent of control cultures, with blank values subtracted. The concentration giving a SI of 50% (IC50) in each cell lines was extracted from dose-response curves by custom-made computer software in Excel (Microsoft Corporation, Redmond, WA). For CEL, Taxol, and paclitaxel the overall mean cell line log10 IC50 was determined de-

TABLE 3
Response Rates (%) in Patient Tumor Samples for the Indicated Drugs and Diagnoses[a]

Tumor type	Taxol 5.0 μg/mL % (no.)	CEL 5.0 μg/mL % (no.)	Vcr 0.5 μg/mL % (no.)	Cisp 2.0 μg/mL % (no.)	Ara-C 0.5 μg/mL % (no.)	VP-16 5.0 μg/mL % (no.)	Dox 0.5 μg/mL % (no.)	4-HC 2.0 μg/mL % (no.)
ALL	55 (31)	73 (41)	78 (41)	84 (31)	73 (41)	85 (41)	82 (40)	89 (37)
AML	61 (36)	61 (41)	43 (37)	30 (37)	71 (38)	71 (38)	55 (36)	42 (36)
NHL	83 (6)	71 (7)	100 (6)	50 (6)	67 (6)	0 (6)	33 (6)	75 (4)
CML	50 (4)	17 (6)	50 (4)	67 (3)	50 (4)	0 (4)	50 (4)	50 (4)
CLL	87 (8)	91 (11)	100 (8)	37 (8)	87 (8)	25 (8)	100 (7)	87 (8)
Ovarian	11 (19)	18 (22)	12 (16)	44 (18)	0 (15)	27 (15)	5 (18)	6 (16)
NSCLC	40 (15)	21 (19)	13 (15)	29 (14)	0 (14)	14 (14)	14 (14)	10 (10)
Breast	57 (7)	43 (7)	0 (2)	71 (7)	0 (2)	0 (4)	29 (7)	14 (7)
Colorectal	0 (5)	14 (7)	0 (5)	0 (5)	0 (4)	0 (5)	0 (5)	0 (3)
Sarcoma	50 (6)	57 (7)	50 (6)	67 (6)	20 (5)	20 (5)	50 (6)	50 (4)
Assorted[b]	55 (9)	23 (13)	33 (9)	50 (8)	22 (9)	37 (8)	44 (9)	33 (6)
Total (hematologic)	60 (87)	67 (108)	66 (98)	54 (87)	72 (99)	66 (99)	69 (95)	68 (91)
Total (solid)	27 (59)	25 (73)	18 (51)	41 (56)	4 (47)	18 (49)	17 (57)	11 (44)
Response ratio (S/H)	0.45	0.37	0.27	0.76	0.06	0.27	0.25	0.16

CEL: Cremophor EL; Vcr: vincristine; Cisp: cisplatin; Ara-C: cytarabine; VP-16: etoposide; Dox: doxorubicin; 4-HC: 4-hydroperoxycyclophosphamide; ALL: acute lymphocytic leukemia; AML: acute myelocytic leukemia; NHL: non-Hodgkin's lymphoma; CML: chronic myelocytic leukemia; CLL: chronic lymphocytic leukemia; NSCLC: nonsmall cell lung carcinoma; S/H: solid/hematologic.

[a] Response rate (RR) is the fraction of samples (%) with survival index below the median for each drug, based on all samples included in the study. The empirically derived cutoff concentrations are indicated for each drug. The CEL concentration is expressed as the corresponding Taxol concentration. The number of samples investigated for each condition are shown within parentheses. The response ratio was obtained by dividing the total RR for the solid samples with that of the hematologic samples.

[b] Assorted tumors include (with numbers in parentheses): carcinomas of the adrenal cortex (2), kidney (1), liver (1), salivary duct (1), and urinary bladder (2), choriocarcinoma (1), gonadal tumor (1), malignant histiocytoma (1), myeloma (1), small cell lung carcinoma (1), and Wilms' tumor (1).

fined as the mean of the log10 values for all cell lines. The overall log 10 IC50 of each cell line was then subtracted from the individual cell line log10 IC50 to yield a variable denoted delta. A mean graph comprised of the deltas for each drug across the cell line panel could then be constructed to visualize differential cytotoxicity patterns of drugs.[23] Thus, positive values indicate cell lines being more resistant than average and negative values indicate cell lines more sensitive than average for a particular drug. The correlations between the log IC50s for various drugs, (i.e., cross-resistance) were determined using Pearson's correlation coefficient.[24]

The results in the patient samples are presented as in vitro response rates, defined as the percentage of samples showing a SI value below the median, calculated from all samples included in the study for that drug at its EDCC. The statistical calculations were made using StatView (Abacus Concepts, Inc., Berkeley, CA).

RESULTS

Cell Lines

The effect on SI of increasing concentrations of Taxol, paclitaxel, and the corresponding concentrations of CEL in the parental cell line U937-GTB and its subline U937-GTB/Vcr is depicted in Figure 1. The parental cell line was highly sensitive to Taxol and paclitaxel, whereas CEL was active only at relatively high concentrations. The subline showed a similar dose-response curve to CEL as the parental line, whereas it was more resistant to Taxol and even more so to paclitaxel, which may indicate a resistance reversal effect of CEL in the Taxol preparation.

Cytotoxic activity, expressed as deltas for CEL, Taxol, and paclitaxel for each cell line, as well as the proposed mechanisms of resistance for the sublines, are shown in Figure 2. CEL showed little differential effect in the cell lines, but was somewhat more active in the resistant 8226/LR-5, H69AR, and ACHN lines. Taxol and paclitaxel showed very similar activity patterns, probably indicating that the active component in both formulations is paclitaxel, with the 8226/Dox40, NCI-H69, and ACHN cell lines being most resistant.

The activity, expressed as IC50s of CEL, paclitaxel, and Taxol is depicted in Table 1. The IC50s for paclitaxel were 2–25 times higher than for Taxol, except in U937-GTB and 8226/S and its subline 8226/LR-5, in which they were equally active. The difference was most pronounced in NCI-H69, followed by U937-Vcr and the P-gp-expressing 8226/Dox40 sublines. The sublines U937-Vcr and 8226/Dox40 were 31 and 700 times more resistant, respectively, to paclitaxel than their parental cell lines, but only 2 and 65 times more resistant to Taxol (Table 1). Conversely, the subline H69AR was 500- and 40-fold more sensitive to paclitaxel and Taxol, respectively, than its parental cell line. In all cell lines, except ACHN and NCI-H69, CEL was considerably less active than Taxol.

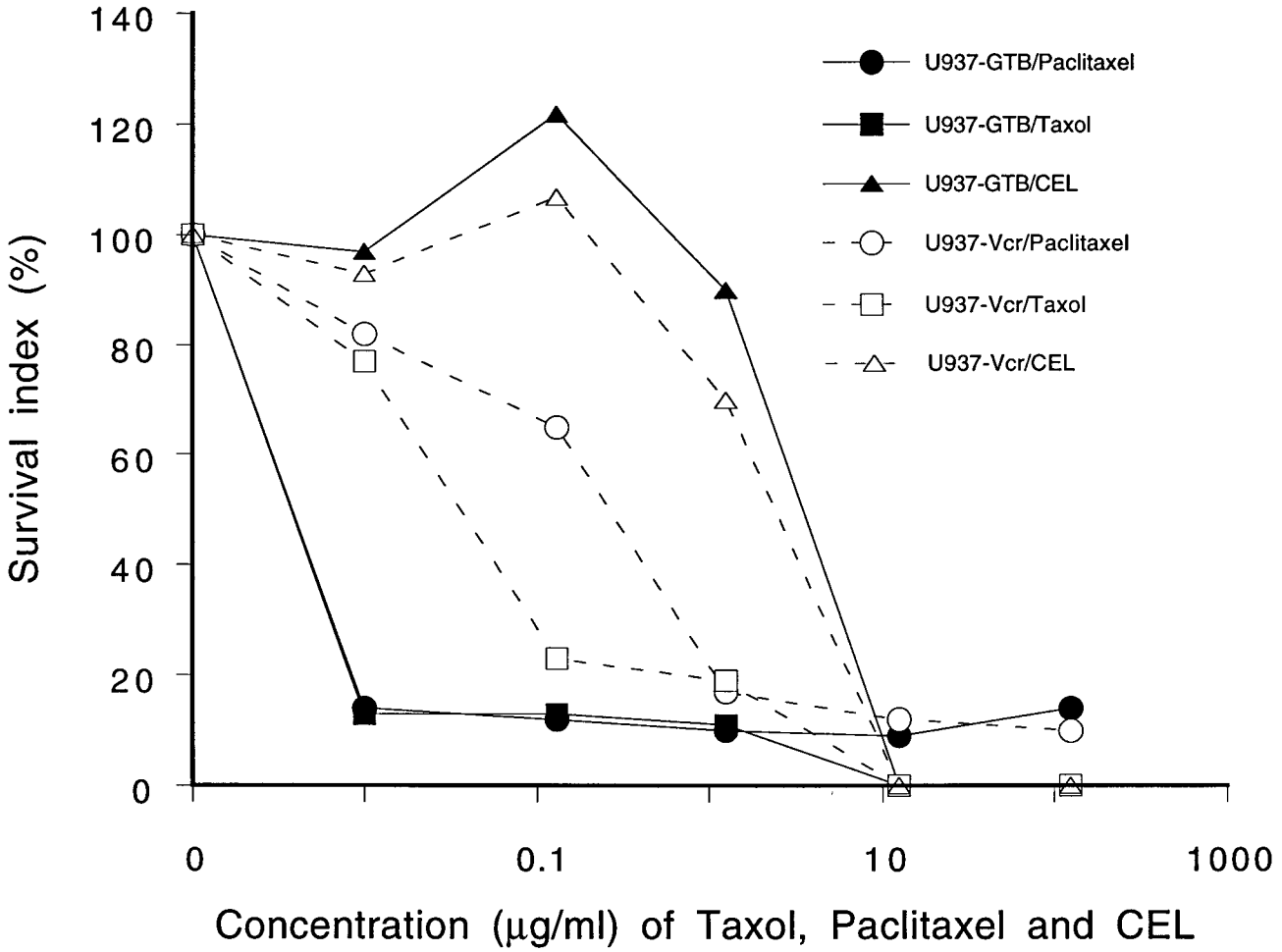

FIGURE 1. Effect on survival index of increasing concentrations of Cremophor EL (CEL), paclitaxel, or Taxol in the U937-GTB cell line (black symbols) and in the resistant subline U937-GTB/Vcr (open symbols). The total solvent CEL concentrations were 0.0, 0.0034, 0.017, 0.083, 0.42, and 2.1%, respectively, for the indicated paclitaxel concentrations. Paclitaxel formulated in ethanol contained the corresponding concentrations of ethanol. One typical experiment is presented.

The cross-resistance between CEL or Taxol and other cytotoxic drugs was evaluated by correlating their log IC50s in all ten cell lines, and the drugs were then ranked according to the correlation coefficient (Table 2). CEL was not cross-resistant to any drug. There was rather a significant inverse relationship to 4-hydroperoxycyclophosphamide (4-HC) Dox, etoposide (VP-16), and Vcr. The similarity between Taxol and paclitaxel was substantiated by the high correlation (0.95) between their log IC50s. Furthermore, Taxol showed significant cross-resistance with six other drugs, five of which were tubulin-active agents.

Patient Cells

The hematologic tumors generally showed high response rates to the cytotoxic drugs, whereas solid tumors were less responsive (Table 3). Colorectal carcinomas responded poorly to the standard drugs, whereas carcinomas of the ovary and breast, sarcomas, and nonsmall cell lung carcinoma had variable, but on average intermediate, sensitivity. This overall pattern was also true for Taxol and CEL, which were similarly active. The highest response rates for CEL and Taxol were among hematologic tumors, and sarcomas and breast carcinoma among solid tumors. The response ratio, obtained by dividing the overall response rate for solid tumors with that for hematologic tumors, was highest for cisplatin (0.76), followed by Taxol (0.45) and CEL (0.37).

The correlation coefficient between the standard drugs and Taxol was ≤ 0.50 in solid as well as hematologic tumor samples, with the exception of VP-16, for which it was 0.67 in solid tumors (Table 4). Between

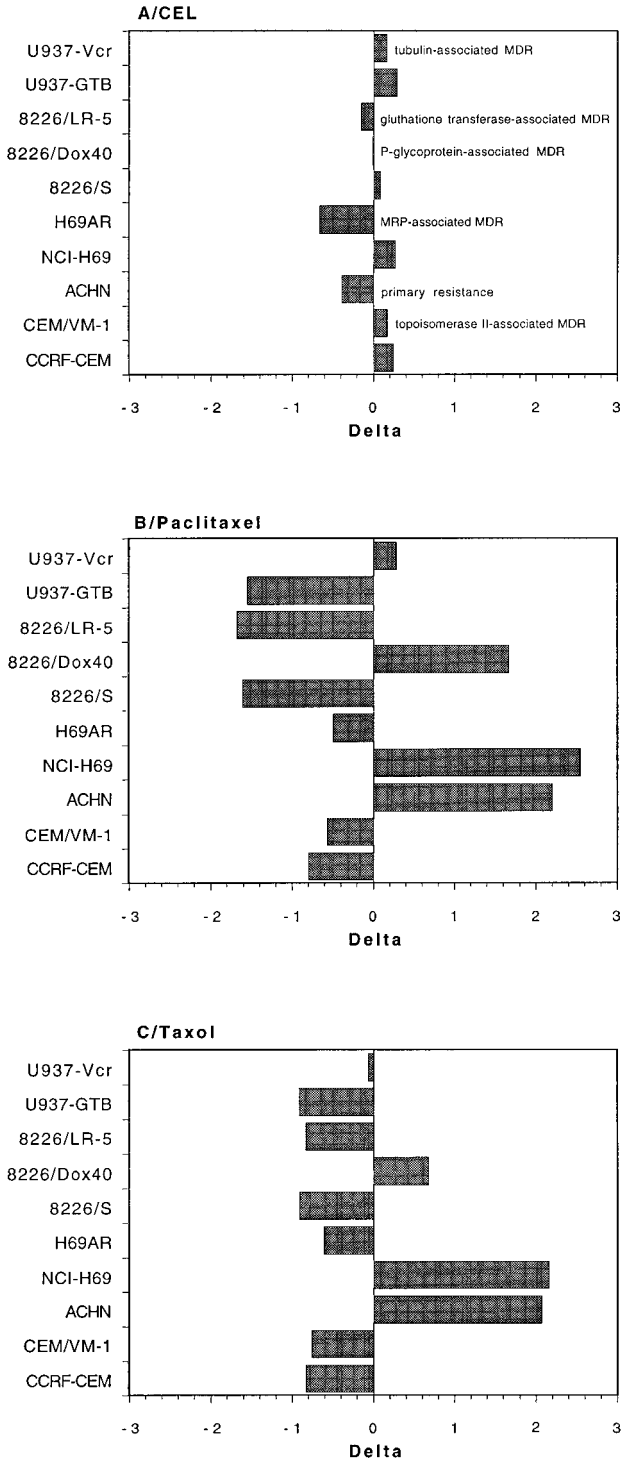

FIGURE 2. Delta, i.e., the log IC50 of a cell line minus the mean log IC50 of all cell lines in the panel for (A) Cremophor EL/ethanol (CEL), (B) paclitaxel in ethanol (paclitaxel), and (C) Taxol. The proposed mechanisms of resistance for the sublines are depicted in A. Positive delta value indicates greater resistance and negative delta value indicates greater sensitivity than average for that particular drug. One typical experiment is presented.

TABLE 4
Cross-Resistance Pattern Expressed as Correlation Coefficients for the Survival Indices between CEL or Taxol and the Indicated Cytotoxic Drugs in Solid and Hematologic Tumor Samples[a]

Drug	Taxol (S)	CEL (S)	Taxol (H)	CEL (H)
Taxol	1.00	0.63	1.00	0.82
Cisp	0.38	0.32	−0.03	−0.18
Ara-C	0.50	0.36	0.07	0.02
VP-16	0.67	0.33	0.09	0.23
Dox	0.40	0.31	0.23	0.17
Vcr	0.46	0.27	0.34	0.40
4-HC	0.41	0.28	0.24	0.26

CEL: Cremophor EL: Cisp: cisplatin; Ara-C: cytarabine; VP-16: etoposide; Dox: doxorubicin; Vcr: vincristine; 4-HC: 4-hydroperoxycyclophosphamide; S: solid; H: hematologic.

[a] Drug concentrations used were the empirically derived cutoff concentrations. Based on data obtained from the tumor samples used in Table 3.

CEL and the standard drugs the correlation coefficient varied between −0.18 and 0.40. Between Taxol and CEL, the cross-resistance was higher, 0.82 and 0.63, for hematologic and solid tumors, respectively (Table 4).

DISCUSSION

In most cell lines paclitaxel and Taxol were both highly active, whereas CEL alone was active only at high concentrations, which is reminiscent of the effect in the P-gp-expressing KB 8-5-11 cell line for various surfactants.[4] The close relationship between paclitaxel and Taxol was indicated by the very high correlation coefficient between these drugs. Furthermore, the high correlations between paclitaxel, irrespective of formulation, and other tubulin-active agents (e.g., colchicine, vincristine, and vinblastine) support the hypothesis that tubulin is the target for paclitaxel as described,[8,25] and also that CEL does not interact with the drug-target interaction in cell lines.

However, in several cell lines, CEL added considerably to the several-fold higher activity of Taxol than paclitaxel, as well as in cell lines not selected for drug resistance. This may be due to a potentiating effect of CEL on paclitaxel, e.g., by reversal of drug resistance. It should be noted that the sublines with the most resistance to paclitaxel compared with their parental lines, U937-Vcr and 8226/Dox40, were among the cell lines in which Taxol was much more active than paclitaxel. This correlates with previous findings of resistance to paclitaxel mediated by P-gp and altered tubulin[25] and adds to these earlier findings by indicating the modulation of P-gp and altered tubulin by CEL.

Conversely, the melphalan- and teniposide-resis-

tant 8226/LR-5 and CEM/VM-1 sublines, respectively, were not cross-resistant to paclitaxel, indicating that increased glutathione-transferase activity and altered topoisomerase II do not alter paclitaxel sensitivity. In agreement with recent reports, the MRP expression of the H69AR subline did not confer paclitaxel resistance.[26,27] On the contrary, this subline showed collateral sensitivity to paclitaxel, the basis for which remains to be elucidated.

In the NCI-H69, 8226/Dox40, and ACHN cell lines CEL was more active than paclitaxel, whereas Taxol was closer to CEL in activity. This pattern is reminiscent of that in the patient samples. The reason for this is unclear but indicates that these cell lines may be used as models in further studies detailing the differential effects of CEL and paclitaxel.

Compared with paclitaxel and Taxol, there was very little differential effect of CEL among the cell lines. This means that the correlations with other drugs, as presented in Table 2, should be interpreted with caution. However, it might be concluded that CEL cytotoxicity is not possible to classify into the standard mechanistic groups.

In tumor cells from patients, Taxol was highly active in hematologic tumors and variably, but mostly less, active in solid tumors (Table 3). Although the clinical pattern of activity for Taxol is far from established, it has been shown to be active in lymphomas, carcinomas of the breast and ovary, and in nonsmall cell lung carcinoma and inactive in colorectal carcinoma.[8,28] With the exception of ovarian carcinoma, the activity pattern obtained in vitro is reminiscent of that in the clinic. In the authors' previous study,[7] the response rate to Taxol for ovarian carcinoma was considerably higher, 43%, compared with 11% in the current study. This difference could perhaps be explained by the inclusion of a higher proportion of clinically ''resistant'' ovarian carcinoma samples in the current study. In addition, the ratio of solid to hematologic tumors in vitro response rates for Taxol was higher (0.45) than for most standard drugs, confirming the belief that Taxol is a drug with relatively high solid tumor activity.

To summarize, the current data indicate the validity of FMCA in the assessment of the activity of standard drugs[20,22] and probably of Taxol as well, although the exact assessment for the latter drug must await the reporting of ongoing studies in, for example, hematologic malignancies.[29]

In patient samples, CEL at a concentration corresponding to that in Taxol produced essentially similar cytotoxic effects as Taxol (Table 3), which was also confirmed in separate control experiments, in which paclitaxel produced very little effect in tumor cells from patients.[7] Taxol and CEL similarity was also confirmed in the cross-resistance analysis (Table 4). Tubulin is one potential cytoplasmic target for anticancer drugs, but the low correlation between Taxol or CEL and Vcr indicates that the Taxol and CEL effects may not be through tubulin interaction in the patient tumor cells in vitro. Conversely, the relatively high correlation between Taxol and CEL (0.82 and 0.63 for hematologic and solid tumors, respectively) suggests that these agents share similar mechanisms of action in vitro. The lack of high cross-resistance between Taxol and standard cytotoxic drugs is compatible with clinical experience.

The current findings lead to the hypothesis that CEL may also contribute to the cytotoxic activity of Taxol in patients. In this context, it is interesting to note that a standard dose of Taxol given in a 3-hour infusion results in a long-lasting plasma concentration of CEL close to that active in the patient samples in the current study, and that is able to modulate P-gp mediated MDR in vitro.[5] Pilot clinical studies on the use of CEL as a resistance modifier and cytotoxic agent are ongoing.[6] Conversely, the findings of a very low distribution volume and tissue accumulation of CEL in mice indicate that CEL would not contribute significantly to the clinical activity of Taxol.[9] However, the distribution of CEL to tumor tissue is unknown. Because there are great differences in many aspects between the in vitro and in vivo milieu, it can not be excluded that the current findings are confined only to the in vitro situation. Ongoing clinical trials using paclitaxel formulated in liposomes[30] may come to elucidate the clinical importance of CEL.

The preclinical development of Taxol relied on various cell lines used in vitro or in vivo as targets for the cytotoxic effect.[8,28] Considering the current results of a generally high activity of paclitaxel formulated as Taxol or in ethanol and a mostly lower CEL effect in cell lines, it was reasonable to conclude that CEL was mainly a passive excipient. Although the clinical activity of CEL in the Taxol formulation has by no means been proven so far, the current data point to the importance of using not only cell lines but also preclinical tumor models with higher clinical validity in new drug development.

Taxol was approximately 5 times less active in patient samples than in cell lines considering the IC50 in cell lines at 1 μg/mL, and an overall cell survival of approximately 50% at 5 μg/mL in patient tumor cells (data not shown). The latter concentration is within the range achievable as the maximum concentration of drug in patients.[8] Furthermore, paclitaxel was active in most cell lines, but not in patient samples, whereas the CEL activity was essentially the same in both mod-

els. The reason for this difference is unknown. However, one apparent difference is the high proliferation rate in cell lines whereas that in tumor cells from patients is very low or nonexistent.

Considering the apparent role of microtubules in mitosis, it appears reasonable that a paclitaxel-induced disruption of this process makes the continuously proliferating cell lines highly sensitive to the drug, whereas in resting cells it may be active only at very high concentrations. This conclusion was partly substantiated by the finding that the paclitaxel-resistant cell lines ACHN, NCI-H69, and 8226/Dox40 were the slowest proliferating cell lines (data not shown).

The starting material for CEL production is castor oil, which is mainly comprised of triglycerides of the unsaturated ricinoleic fatty acid.[4] Fatty acids have been found to be cytotoxic against tumor cells and the mechanism may be the generation of free radicals by peroxidation of polyunsaturated fatty acids and/or a direct perturbing effect in the cell membrane causing increasing fluidity and leakage.[31–33] In light of these observations and the current findings, it may prove worthwhile to better define the active component of CEL and related surfactants for use as lead molecules in cytotoxic drug development.

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