In Cancer Epidemiology and Prevention (Schottenfeld D, Fraumeni JF Jr, eds). Philadelphia: Saunders, 1982, pp 855–870
(*23*) Sievert RM: A circulating physical department for standardising the roentgen radiation used in therapy. Acta Radiol 5:457–467, 1926
(*24*) Thoraeus R: Features of the system of periodical inspection adopted for roentgen therapy installations in Sweden, and some experiences of the inspection work. Acta Radiol 33:253–280, 1950
(*25*) Mattsson B, Rutqvist L-E: Some aspects on validity of breast cancer, pancreatic cancer and lung cancer registration in Swedish official statistics. Radiother Oncol 4:63–70, 1985
(*26*) Committee on the Biological Effects of Ionizing Radiations Health Effects of Exposure to Low Levels of Ionizing Radiation. BEIR V. Washington, DC: Natl Acad Press, 1990
(*27*) Boice JD Jr, Harvey EB, Blettner M, et al: Cancer in the contralateral breast after radiotherapy for breast cancer. N Engl J Med 326:781–785, 1992
(*28*) Storm HH, Andersson M, Boice JD Jr, et al: Adjuvant radiotherapy and risk of contralateral breast cancer. J Natl Cancer Inst 84 1245–1250, 1992
(*29*) Hrubec Z, Boice JD Jr, Monson RR, et al Breast cancer after multiple chest fluoroscopies: second follow-up of Massachusetts women with tuberculosis. Cancer Res 49:229–234, 1989

Notes

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Measurement of Cremophor EL Following Taxol: Plasma Levels Sufficient to Reverse Drug Exclusion Mediated by the Multidrug-Resistant Phenotype

*Lorraine Webster, Martha Linsenmeyer, Michael Millward, Carmel Morton, James Bishop, David Woodcock**

Background: **Paclitaxel (Taxol) is the first of a new class of cytotoxic agents with activity against tumors resistant to other drugs. For clinical use, paclitaxel is currently formulated in a vehicle of 50% ethanol and 50% polyethoxylated surfactant Cremophor EL (Cremophor). We have previously shown that Cremophor will block the P-glycoprotein drug efflux pump responsible for the multidrug-resistant phenotype. Overexpression of P-glycoprotein is one mechanism of in vitro resistance to a number of currently used cytotoxic agents including paclitaxel. *Purpose:* Our aim was to develop a bioassay to measure plasma levels of Cremophor and to determine whether or not plasma levels of Cremophor achieved during paclitaxel therapy are sufficient to inhibit the activity of the P-glycoprotein. *Methods:* All patients studied had histologically proven, advanced ovarian carcinoma with measurable or evaluable disease and had received at least one prior platinum-containing regimen. The bioassay used flow cytometry to measure the increase in equilibrium intracellular daunorubicin levels in multidrug-resistant human T-cell leukemia cells (CEM/VLB_{100}) in the presence of a series of concentrations of Cremophor. Levels of Cremophor were measured in plasma from 21 patients after a 3-hour infusion of 135 or 175 mg/m² paclitaxel. Both dose levels were given following premedication with oral dexamethasone, intravenous promethazine hydrochloride, and intravenous cimetidine. The Cremophor bioassay involved incubation of CEM/VLB_{100} cells (5×10^5) for 1 hour with 2 μg/mL daunorubicin in 0.5 mL HL-1 medium plus 0.5 mL plasma prior to flow cytometric analysis. Pretreatment plasma was used to derive a standard curve for the effect of Cremophor on equilibrium daunorubicin levels. All measurements were done in triplicate. *Results:* In vitro experiments indicated that, for maximal inhibition of P-glycoprotein activity, concentrations of Cremophor of 0.1% (vol/vol) were required. At the end of a 3-hour infusion of paclitaxel, plasma levels of Cremophor in 19 of 21 patients were 0.1% or higher and 0.09% in the remaining two. Concentrations of 5-20 μ*M* paclitaxel dissolved in ethanol without Cremophor did not inhibit P-glycoprotein in this assay. *Conclusion:* The concentrations of Cremophor measured in plasma drawn from patients after a 3-hour infusion of paclitaxel at 135 or 175 mg/m² were found to be sufficient to inhibit P-glycoprotein activity in vitro. *Implications:* The efficacy of paclitaxel against some tumors may be aided by its administration in a vehicle solution containing Cremophor in quantities that reach concentrations in the plasma sufficient to reverse multidrug resistance of neoplastic cells. [J Natl Cancer Inst 85:1685–1690, 1993]**

Multidrug resistance is a mechanism of cellular resistance to chemotherapy in which tumor cells express elevated levels of a membrane transport protein, the P-glycoprotein, that actively pumps out of the cell a broad spectrum of structurally unrelated drugs (*1*). Bell et al. (*2*) first reported high levels of P-glycoprotein in tumor samples from two of five patients with advanced drug-resistant ovarian cancer. Subsequently, the potential importance of multidrug resistance in clinical resist-

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ance to chemotherapy has been demonstrated in other tumors, including hematologic malignancies (*3,4*), breast cancer (*5*), childhood sarcomas (*6*), and lung cancer (*7,8*).

Paclitaxel (Taxol) is a microtubule-stabilizing drug that appears to be the most active single agent identified to date against a number of tumors [reviewed in (*9*)]. In phase II studies (*9*), response rates of 30% have been reported in previously treated ovarian cancer and up to 60% in advanced breast cancer. In addition, the activity of paclitaxel against untreated non–small-cell lung cancer is greater than that of other agents (*9*). Although the antitumor activity of *Taxus brevifolia* extracts was first identified in 1963 and the structure of the active component was reported in 1971 (*10*), the development of this agent has been delayed in part because of its lack of clearly superior activity compared to the activity of other agents and because of its low aqueous solubility (*9*). In the current clinical formulation, paclitaxel is dissolved in a mixture of 50% ethanol and 50% Cremophor EL (Cremophor), such that most patients receive more than 20 mL Cremophor with each dose of paclitaxel. Cremophor is a polyethoxylated castor oil that is used as a solubilizing agent for several other drugs including teniposide, miconazole, cyclosporine, and some vitamin preparations. Cremophor has been implicated as a cause of the hypersensitivity reactions seen in early clinical trials of paclitaxel (*11*). However, prolongation of the infusion time and administration of prophylactic antiallergy medications have reduced the occurrence of this side effect (*11*).

Like a number of other polyethoxylated surfactants (*12-14*), Cremophor inhibits the drug efflux activity of the P-glycoprotein pump in vitro (*14-16*) and increases the sensitivity of a multidrug-resistant murine transplantable tumor to doxorubicin in vivo (*12*). Paclitaxel is also one of the diverse group of drugs to which multidrug-resistant cells are cross-resistant (*17,18*). Therefore, paclitaxel formulated in a Cremophor solution consists of both the cytotoxic agent and a compound potentially capable of overcoming resistance to paclitaxel.

Because of the chemical inertness and lack of spectral properties of Cremophor, we have developed a bioassay that has enabled us to measure the concentration of Cremophor in plasma and to determine the levels required for reversal of multidrug resistance in vitro. The assay is based on the ability of Cremophor to act as a multidrug resistance-reversing agent and, thus, to prevent the exclusion of a fluorescent drug that is pumped out of cells by the P-glycoprotein. The assay was then used to measure Cremophor in plasma from patients with advanced ovarian cancer who received a 3-hour infusion of paclitaxel.

Patients and Methods

Study Design

Twenty-one patients eligible to receive paclitaxel for ovarian cancer were studied (*see* Table 1). Patients were required to have histologically confirmed advanced ovarian carcinoma with measurable or evaluable disease and to have received at least one prior platinum-containing regimen. Other prior chemotherapy up to a maximum of three regimens and prior radiotherapy were permitted. Other eligibility criteria were age of 75 years or less, a performance status of 0-2 according to the Eastern Cooperative Oncology Group criteria, and adequate baseline values of hematologic (absolute neutrophil count $\geq 2.0 \times 10^9$/L and platelet count $\geq 100 \times 10^9$/L), renal (serum creatinine level $\leq 1.5 \times$ upper limit of normal), and hepatic (serum bilirubin level $\leq 1.25 \times$ upper limit of normal) function.

Patients who had one or two previous chemotherapy regimens received 175 mg/m^2 paclitaxel (Taxol; Bristol-Myers Squibb, Melbourne, Australia), and patients with three prior regimens received 135 mg/m^2. Both dose levels were given as a 3-hour infusion following premedication with oral dexamethasone, intravenous promethazine hydrochloride, and intravenous cimetidine. Paclitaxel was supplied as a 6-mg/mL solution in 50% Cremophor and 50% ethanol, and the appropriate dose was diluted in a 1000-mL 5% dextrose solution for infusion.

On the first cycle of paclitaxel, blood samples (10-20 mL) were drawn into tubes containing lithium heparin prior to commencement of the infusion and upon completion of the infusion. Plasma was separated and frozen at –70 °C until analysis. Written informed consent was obtained for administration of paclitaxel and taking of blood samples. The protocol was approved by the institutional ethics committee.

Cremophor Bioassay

Multidrug-resistant CEM/VLB$_{100}$ cells (derived from the human CCRF-CEM T-cell leukemia) (*19*) were maintained in the α-modification of Eagle's minimum essential medium with 10% newborn calf serum (Cytosystems, Castle Hill, Australia) and 100 ng/mL vinblastine (David Bull Laboratories, Melbourne). These cells had been designated as "R100 cells" in previous publications (*4,12,14*). Serum-free, phenol red-free HL-1 medium was obtained from Ventrex Laboratories, Portland, Maine. Cremophor EL was from BASF Fine Chemicals, Melbourne.

The method for determining the activity of the P-glycoprotein drug efflux pump has been previously described (*12,14*). Briefly, 5×10^5 cells in logarithmic growth were incubated at 37 °C in 1 mL medium with 2 μg/mL daunorubicin (David Bull Laboratories) for 1 hour, during which time intracellular drug levels reached equilibrium. The effect of Cremophor on the activity of the P-glycoprotein drug efflux pump was rapid, since preincubation with Cremophor had no additional effect on equilibrium daunorubicin levels (*12*). Cells were then analyzed for intracellular daunorubicin fluorescence by flow cytometry using a FACStar Plus cell sorter (Becton Dickinson, Mountain View, Calif.). All measurements were done in triplicate. The units of fluorescence were arbitrary, and absolute values from different days cannot be directly compared. Studies were done to determine the effect of serum on the activity of Cremophor, to ascertain whether plasma could be substituted for serum, and to assess the effect of freezing on plasma samples containing Cremophor.

To measure Cremophor in plasma from patients, we incubated CEM/VLB$_{100}$ cells (5×10^5) for 1 hour with 2 μg/mL daunorubicin in 0.5 mL HL-1 medium plus 0.5 mL plasma. All measurements were done in triplicate. Pretreatment plasma was used to derive a standard curve for the effect of Cremophor on equilibrium daunorubicin levels. The components in each assay were added in the following order: plasma (Cremophor, diluted in HL-1 medium for the standard curves only), CEM/VLB$_{100}$ cells in HL-1 medium, and daunorubicin. The increase in equilibrium intracellular daunorubicin levels in assays containing post-treatment plasma gave a measure of the Cremophor concentration.

Results

Assay Development

Because Cremophor interacts with components of human plasma (*20*), we investigated the effect of increasing amounts of serum and plasma on the Cremophor-induced increase in equilibrium intracellular levels of daunorubicin, a drug excluded by multidrug-resistant cells, such as the CEM/VLB$_{100}$ cells used in this assay. When the artificial serum-free HL-1 medium (<30 μg/mL of total protein) was used, the maximum increase in intracellular

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Table 1. Cremophor plasma levels in patients with advanced ovarian cancer immediately following a 3-hour infusion of paclitaxel

Patient No.	Age, y	Previous therapy*	Paclitaxel dose, mg†	Cremophor dose, mL‡	Plasma Cremophor (% v/v)
1	39	Cisplat + Cyclo	278	23.2	0.20
2	48	Cisplat + cyclo; Carbo + Cyclo, Carbo	243	20.3	0.14
3	58	Cisplat + Epi	290	24.2	0.14
4	60	Carbo	270	22.5	0.15
5	59	Chloram; Carbo; Carbo + ADR	202	16.8	0.17
6	54	Carbo + Cyclo; Cisplat + VP16	324	27.0	0.17
7	45	Carbo/cyclo; Cisplat	313	26.1	0.14
8	55	Melphalan; Cisplat + VP16 + Bleo	280	23.3	0.14
9	54	Cisplat + Cyclo	320	26.7	0.11
10	30	Carbo + Cyclo; HMM; Cisplat	277	23.1	0.14
11	65	Carbo + Cyclo; Chloram	305	25.4	0.15
12	61	Carbo + Cyclo; Carbo	303	25.3	0.09
13	60	Cisplat + Cyclo; Carbo	302	25.2	0.15
14	52	Carbo + Cyclo; Epi + Ifos	285	23.8	0.12
15	57	Carbo + Cyclo	310	25.8	0.09
16	57	Cisplat + Cyclo	306	25.5	0.15
17	67	Cisplat + Cyclo; Mitox + MMC; Cisplat + Cyclo	232	19.3	0.11
18	43	Carbo + Cyclo; Chloram	216	18.0	0.13
19	54	Cisplat + Cyclo; Carbo	256	21.3	0.11
20	55	Cisplat + Cyclo	277	23.1	0.17
21	46	Cisplat + Cyclo, Cisplat + VP16	265	22.1	0.18

*ADR = doxorubicin; Bleo = bleomycin; Carbo = carboplatin; Chloram = chlorambucil, Cisplat = cisplatin; Cyclo = cyclophosphamide; Epi = epirubicin, HMM = altretamine, Ifos = ifosfamide; Mitox = mitoxantrone; MMC = mitomycin; and VP16 = etoposide.

†The paclitaxel dose was 175 mg/m² except in four patients (2, 5, 17, and 18), who received 135 mg/m².

‡The Cremophor dose was calculated from the paclitaxel dose administered.

daunorubicin occurred at a Cremophor concentration of 0.1%, although 0.01% was almost equally effective (Fig. 1). When the HL-1 medium was supplemented with 25% newborn calf serum, 0.1% Cremophor was still required for the maximum effect, but 0.01% had little effect. Increasing the proportion of serum to 50% or 75% produced curves similar to those found in the presence of 25% serum. It was not possible to use 100% serum because of problems with viscosity. Although the maximum intracellular daunorubicin fluorescence for cells in HL-1 medium was higher in the absence of serum, the absolute percentage increase above the background fluorescence was similar under both conditions. Human serum and plasma gave essentially identical results (data not shown). For convenience, plasma was used in all further studies.

The effect of Cremophor on intracellular daunorubicin levels was not related to altered distribution of the drug, as reported to be the case for multidrug-resistant cells treated with cyclosporine (*21*). The localization of daunorubicin in the CEM/VLB_{100} cells (and in its drug-sensitive parent, CCRF-CEM cells) remained principally nuclear with or without Cremophor, the only difference being that intracellular fluorescence increased markedly in the multidrug-resistant cell type when Cremophor was present (data not shown).

To determine whether paclitaxel itself could alter daunorubicin fluorescence in this assay, we dissolved paclitaxel (pure substance) in ethanol at 0.5 m*M* and then diluted it in HL-1 medium. Paclitaxel at 5, 10, or 20 μM—concentrations that are in the range of reported peak levels following 6-hour infusions (*22*)—did not increase intracellular daunorubicin levels. In addition, plasma taken from three pa-

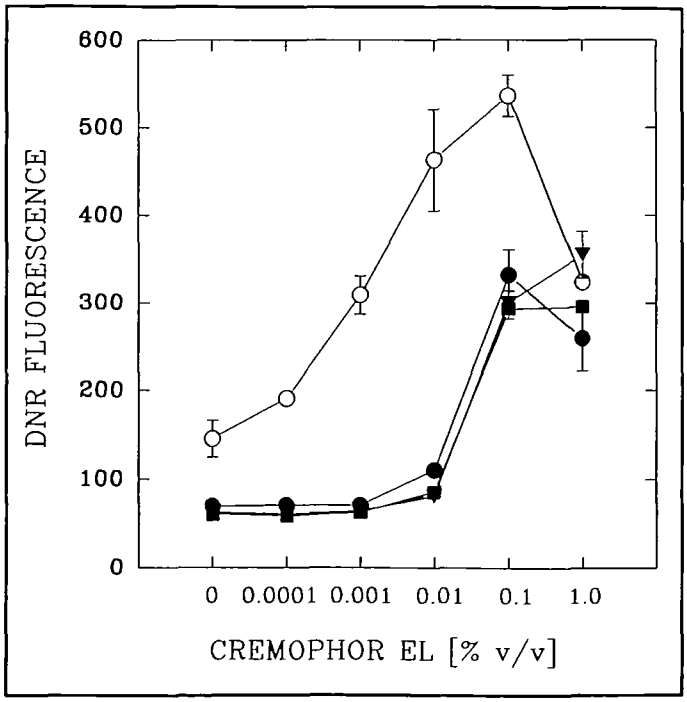

Fig. 1. Multidrug-resistant CEM/VLB_{100} cells were incubated with daunorubicin (DNR) plus a series of concentrations of Cremophor either in HL-1 serum-free medium (○) or in HL-1 medium supplemented with 25% (●), 50% (■), or 75% (▼) newborn calf serum. All samples were run in triplicate, and the vertical bars indicate the standard deviation of the mean of each point. If no bars are apparent, the standard deviation was less than the size of the point.

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tients immediately following intravenous doxorubicin did not increase equilibrium daunorubicin levels above the background.

For use as quality controls in subsequent assays and for the investigation of the effects of freezing plasma containing Cremophor, normal plasma was spiked with three concentrations of Cremophor (0.02%, 0.06%, and 0.2% in the plasma, yielding final assay concentrations of 0.01%, 0.03%, and 0.1%, respectively). The plasma was then frozen in aliquots. When compared to Cremophor standard curves prepared freshly using the same plasma, the plasma frozen with Cremophor gave daunorubicin fluorescence values that differed by –8% to +16% for the 0.1% Cremophor samples. When plasma from six volunteers was tested against this standard curve, differences up to 30% were observed at the 0.1% Cremophor level. To control for this interindividual variability, it is necessary to construct standard curves using each individual's plasma.

Cremophor Concentrations in Plasma From Paclitaxel-Treated Patients

Four patients received 135 mg/m² paclitaxel over a 3-hour period, and 17 patients received 175 mg/m² paclitaxel over a 3-hour period. No hypersensitivity reactions were observed.

Using the described assay, all pretreatment plasma samples (without added Cremophor) gave consistently low intracellular daunorubicin levels equivalent to those observed with plasma from healthy volunteers. Pretreatment plasma was used to construct a standard curve for each of the patients. Data from the first three such assays are presented in Fig. 2. Comparison with Fig. 1 indicates that the concentration-dependent increases in intracellular daunorubicin were very similar for both 50% newborn calf serum and 50% human plasma. The Cremophor concentrations in plasma taken from these patients at the conclusion of the paclitaxel infusion could be read directly from their individual standard curves (Fig. 2). The actual Cremophor levels in the patients' plasma were twice the values read from the standard curve, since the incubation mixtures always contain 0.5 mL plasma and 0.5 mL HL-1 medium. In these patients, post-treatment plasma samples diluted one fifth with pretreatment plasma were also included in the assay. The resulting values read off the standard curve were consistent with a one-fifth dilution of Cremophor (data not shown). Estimates of plasma Cremophor concentration for blood samples taken at the conclusion of the paclitaxel infusion for 21 patients are presented in Table 1. At the end of the paclitaxel infusion, the plasma concentrations of Cremophor were 1.0% or higher for 19 of the 21 patients and 0.9% for the remaining two.

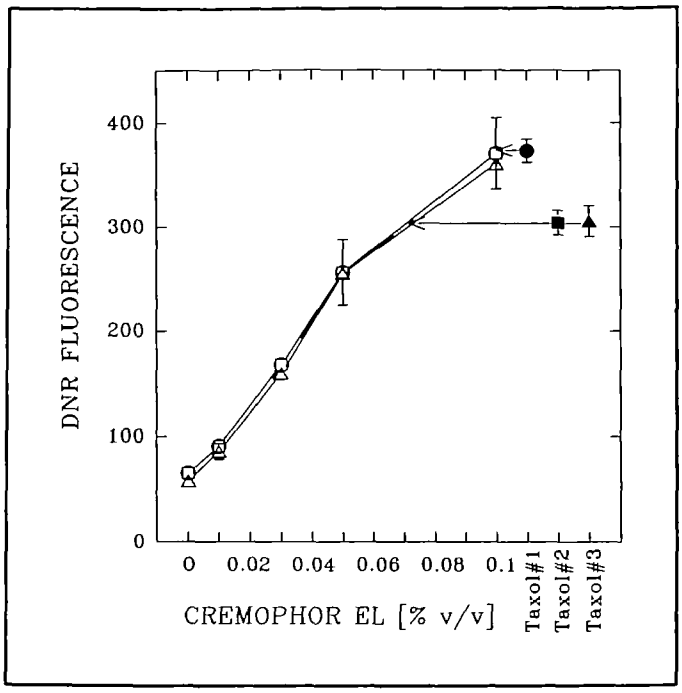

Fig. 2. Percent of Cremophor versus equilibrium daunorubicin (DNR) fluorescence as measured by flow cytometry. CEM/VLB$_{100}$ cells were incubated with 2 μg/mL daunorubicin in 0.5 mL medium and 0.5 mL plasma. Three standard curves (open symbols) were prepared by adding Cremophor to pretreatment patient plasma. Plasma from these patients taken at the end of a 3-hour paclitaxel infusion was also assayed (solid symbols). The Cremophor concentration (contributed by 0.5 mL of plasma in each 1 mL incubation) can be read against the corresponding standard curve (arrows). Cremophor levels in patient plasma are thus twice the value read from the standard curve. All points are means and standard deviations of triplicate measurements.

Discussion

The ability of Cremophor to inhibit P-glycoprotein in vitro has been previously reported (*12,14-16*). Although the interaction of Cremophor with serum components increases the concentration of Cremophor required for this reversal of multidrug resistance, we have demonstrated in vitro activity even in the presence of 75% serum. The interactions between Cremophor, plasma components, and cells are likely to be complex. However, as a deliberate oversimplification, these interactions should be described by the following equations:

$$[\text{Cremophor}] + [\text{plasma}] \leftrightarrow [\text{Cremophor} - \text{plasma}] \quad [1]$$

$$[\text{Cremophor}] + [\text{P-glycoprotein}] \leftrightarrow [\text{Cremophor} - \text{P-glycoprotein}] \quad [2]$$

$$[\text{Cremophor} - \text{plasma}] + [\text{P-glycoprotein}] \leftrightarrow [\text{Cremophor} - \text{P-glycoprotein}] + [\text{plasma}] \quad [3]$$

$$[\text{Cremophor} - \text{plasma}] + [\text{P-glycoprotein}] \leftrightarrow [\text{plasma} - \text{Cremophor} - \text{P-glycoprotein}] \quad [4]$$

In the above equations, "plasma" indicates probable multiple serum/plasma components that interact with Cremophor (*20*), and equilibria involving the "P-glycoprotein" could involve either direct interactions between Cremophor and P-glycoprotein or indirect interactions mediated via effects on the structural organization of the cell membrane. The equilibria involved must be rapid because Cremophor-mediated inhibition of the P-glycoprotein drug efflux pump is the same, regardless of whether the Cremophor had been allowed to form a complex with serum components for 1 hour before daunorubicin was added or whether the Cremophor was added at the same time

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as the daunorubicin (*12*). Similarly, the effect of Cremophor on P-glycoprotein activity (and presumably its interaction with cells) is also readily reversible (*12*). The observation (Fig. 1) that 25%, 50%, or 75% newborn calf serum (or plasma) has a similar effect on the concentration dependency of Cremophor on P-glycoprotein activity would suggest that there is an excess of the component(s) that interact with Cremophor in assays with even 25% serum. The equilibrium (equation 1) is thus driven toward complex formation at all the serum or plasma concentrations tested. In the absence of plasma (HL-1 medium only), most of the change in intracellular daunorubicin concentration occurs over a 100-fold change in Cremophor concentration. This observation would be consistent with an interaction that is primarily bimolecular (equation 2). By contrast, in the presence of serum, only a 10-fold change in Cremophor concentration is required for the bulk of the change in intracellular daunorubicin levels to occur. While extrapolation from simple equilibria should be made with caution, this finding could suggest that, in the presence of serum, the Cremophor interaction with cells is of a cooperative nature (equilibrium in equation 4). However, whatever the nature of the interactions involved, our results suggest that a concentration of 0.1% μL/mL Cremophor in body fluids should be sufficient to inhibit the P-glycoprotein drug efflux pump in tumors expressing the multidrug-resistant phenotype.

The bioassay described here allows the estimation of absolute values of Cremophor concentrations in plasma or serum. While serum components affect the concentration dependency of the Cremophor effect, the equilibrium amounts of intracellular daunorubicin as read from the assay curves relate directly to absolute amounts of Cremophor added to each assay standard. In samples taken from 21 patients who received a 3-hour paclitaxel infusion, there was a sufficiently high circulating level (close to or greater than 0.1% [vol/vol]) of Cremophor in plasma at the end of the infusion for the effective inhibition of action of the P-glycoprotein drug efflux pump. These data do not directly address the question of the relative contribution of the multidrug resistance-reversing surfactant Cremophor in the paclitaxel formulation to the overall efficacy of paclitaxel. The pharmacokinetics of Cremophor in humans are unknown. However, a study performed with rats showed that most of the Cremophor had been cleared by 24 hours, the first time point used (*23*). The activity of paclitaxel when given as a 24-hour infusion, where Cremophor levels are likely to be much lower than following a 3-hour infusion, indicates that high plasma Cremophor levels are not absolutely required for clinical antitumor activity. Further investigation is needed on the relationship between tumor expression of P-glycoprotein, Cremophor levels, and subsequent response to paclitaxel.

Taxotere is the second taxane to enter clinical trials. Early clinical results indicate that the spectrum of antitumor activity of taxotere is likely to be similar to that of paclitaxel (*24*). While not formulated in Cremophor, taxotere is dissolved in polysorbate 80 (Tween 80), another polyethoxylated surfactant that can reverse multidrug resistance in vitro (*12,16*).

The data presented here demonstrate that a Cremophor dose of 16.8-27 mL given over a 3-hour period can produce plasma levels between 0.09% and 0.2%. These levels should be sufficient for in vivo reversal of drug exclusion in cells exhibiting the multidrug-resistant phenotype. Hence, unlike the results with other agents such as verapamil (*25*), which have been administered in conjunction with cytotoxic agents in attempts to overcome multidrug resistance, sufficient circulating levels of the multidrug resistance-reversing agent, Cremophor EL, can be achieved without dose-limiting toxic effects and without the potential hazards of using an immunosuppressive drug such as cyclosporine for this purpose (*26*). Cremophor, therefore, could be investigated as a multidrug resistance modulator with other cytotoxic agents in other schedules.

References

(*1*) Bradley G, Juranka PF, Ling V: Mechanism of multidrug resistance. Biochim Biophys Acta 948:87–128, 1988

(*2*) Bell DR, Gerlach JH, Kartner N, et al: Detection of P-glycoprotein in ovarian cancer: a molecular marker associated with multidrug resistance. J Clin Oncol 3:311–315, 1985

(*3*) Salmon SE, Grogan TM, Miller T, et al: Prediction of doxorubicin resistance in vitro in myeloma, lymphoma, and breast cancer by P-glycoprotein staining. J Natl Cancer Inst 81:696–701, 1989

(*4*) Linsenmeyer ME, Jefferson S, Wolf M, et al: Levels of expression of the *mdr*1 gene and glutathione *S*-transferase genes 2 and 3 and response to chemotherapy in multiple myeloma. Br J Cancer 65.471–475, 1992

(*5*) Sanfilippo O, Ronchi E, De Marco C, et al: Expression of P-glycoprotein in breast cancer tissue and in vitro resistance to doxorubicin and vincristine. Eur J Cancer 27:155–158, 1991

(*6*) Chan HSL, Thorner PS, Haddad G, et al: Immunohistochemical detection of P-glycoprotein: prognostic correlation in soft tissue sarcoma of childhood. J Clin Oncol 8.689–704, 1990

(*7*) Volm M, Mattern J, Samsel B: Overexpression of P-glycoprotein and glutathione *S*-transferase-pi in resistant non-small cell carcinomas of smokers. Br J Cancer 64:700–704, 1991

(*8*) Holzmayer TA, Hilsenbeck S, Von Hoff DD, et al: Clinical correlates of MDR1 (P-glycoprotein) gene expression in ovarian and small-cell lung carcinomas. J Natl Cancer Inst 84:1486–1491, 1992

(*9*) Rowinsky EK, Onetto N, Canetta RM, et al: Taxol: the first of the taxanes, an important new class of antitumor agents. Semin Oncol 19:646–662, 1992

(*10*) Wani MC, Taylor HL, Wall ME, et al: Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia* J Am Chem Soc 93:2325–2327, 1971

(*11*) Peereboom DM, Donehower RC, Eisenhower EA, et al: Successful re-treatment with taxol after major hypersensitivity reactions. J Clin Oncol 11:885–890, 1993.

(*12*) Woodcock DM, Linsenmeyer ME, Chojnowski G, et al: Reversal of multidrug resistance by surfactants. Br J Cancer 66:62–68, 1992

(*13*) Coon JS, Knudson W, Clodfelter K, et al: Solutol HS 15, nontoxic polyethylene esters of 12-hyroxystearic acid, reverses multidrug resistance. Cancer Res 51:897–902, 1991

(*14*) Woodcock DM, Jefferson S, Linsenmeyer ME, et al: Reversal of the multidrug resistance phenotype with Cremophor EL, a common vehicle for water-insoluble vitamins and drugs. Cancer Res 50:4199–4203, 1990

(*15*) Schuurhuis GJ, Broxterman HJ, Pinedo HM, et al: The polyoxyethylene castor oil Cremophor EL modifies multidrug resistance. Br J Cancer 62:591–594, 1990

(*16*) Friche E, Jensen PB, Sehested M, et al: The solvents Cremophor EL and Tween 80 modulate daunorubicin resistance in the *multidrug resistant Ehrlich ascites tumor.* Cancer Commun 2:297–303, 1990

(*17*) Horwitz SB, Lothstein L, Manfredi JJ, et al: Taxol: mechanisms of action and resistance. Ann N Y Acad Sci 466:733–744, 1986

(*18*) Waud WR, Gilbert KS, Harrison SD Jr, et al: Cross-resistance of drug-resistant murine P388 leukemias to taxol in vivo. Cancer Chemother Pharmacol 31:255–257, 1992

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Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

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Sync your system to PACER to automate legal marketing.