Phase I Trial of Cremophor EL with Bolus Doxorubicin

Michael J. Millward, Lorraine K. Webster, Danny Rischin, Kerrie H. Stokes, Guy C. Toner, James F. Bishop, Ian N. Olver, Bernadette M. Linahan, Martha E. Linsenmeyer, and David M. Woodcock

ABSTRACT

Cremophor EL (cremophor), a component of the paclitaxel formulation, can potentially reverse P-glycoprotein-associated multidrug resistance. A Phase I trial of cremophor as a 6-h infusion every 3 weeks was performed with bolus doxorubicin (50 mg/m²). The cremophor dose was escalated from 1 to 60 ml/m². A standard paclitaxel premedication was given before cremophor. Using a bioassay, potentially active cremophor levels (≥1 µl/ml) were measured in plasma from patients receiving cremophor doses of 30, 45, and 60 ml/m². A cross-over design was used to assess the influence of cremophor 30 ml/m² on the pharmacokinetics of doxorubicin and doxorubicinol. The plasma area under the concentration versus time curve (AUC) of doxorubicin increased from 1448 ± 350 to 1786 ± 264 ng/mlh (P = 0.02) in the presence of cremophor, whereas the AUC of doxorubicinol increased from 252 ± 104 to 486 ± 107 ng/mlh (P = 0.02). This pharmacokinetic interaction was associated with significantly increased neutropenia. With reduction of the doxorubicin dose to 35 mg/m², the cremophor dose was increased to 60 ml/m². Dose-limiting toxicities occurred in two of six patients after 45 ml/m² and two of four patients after 60 ml/m², which included febrile neutropenia and grade III cremophor-related toxicities of rash, pruritus, headache, and hypotension. All patients who received 45 ml/m² cremophor reached plasma levels ≥1.5 µg/ml, but at 60 ml/m², only two of four reached this level, and the calculated plasma clearance of cremophor was significantly faster at this dose. One patient with hepaticosa resistant to epirubicin achieved a near-complete response. Cremophor 45 ml/m² over 6 h with 35 mg/m² doxorubicin is recommended for further studies. The pharmacokinetic interaction between cremophor and doxorubicin is quantitatively similar to that described in trials of paclitaxel with doxorubicin and suggests that the cremophor in the paclitaxel formulation is responsible.

INTRODUCTION

The development of resistance to anticancer chemotherapy is a major reason for the occurrence of treatment failure in the management of patients with malignant disease. Although a number of potential mechanisms of such drug resistance have been described, the most widely studied is that of MDR related to the overexpression of a surface glycoprotein termed P-gp (1, 2). P-gp-associated MDR confers resistance in vitro to several important classes of anticancer drugs including anthracyclines, Vinca alkaloids, epipodophyllotoxins, and taxoids (3, 4). Analysis of human tumor samples for the presence of P-gp-associated MDR with a variety of techniques have shown its occurrence in both previously untreated and chemotherapy-refractory patients and its relationship between failure to respond to chemotherapy and poor prognosis (5–8).

Reversal of P-gp-associated MDR in vitro can be achieved with a variety of compounds. Initial clinical trials of these modulators were performed with drugs developed for other clinical uses, for example verapamil, tamoxifen, and cyclosporin A (9–14). Although some promising results were achieved, suggesting the ability of such modulators to enhance the response of tumors to coadministered chemotherapy, toxicity to normal tissues generally prevented the administration of doses necessary for MDR reversal in vitro. Current interest has focused on the development of analogues having potentially less toxicity and the investigation of novel compounds as MDR modulators (15–18).

Cremophor (polyoxyethylenglycoltriricinoleat 35; BASF) is a nonionic solubilizer and emulsifier that is made by reacting ethylene oxide with castor oil (19). Castor oil is primarily a triglyceride of 12-hydroxy-oleic acid, and in cremophor, 35 moles of ethylene oxide are reacted per mole of triglyceride. Cremophor is a pale yellow, oily liquid consisting of a mixture of components, primarily polyethylene glycol conjugates of this triglyceride. Fatty acid esters of polyethylene glycol are also present, as well as hydrophilic polyethylene glycols and ethoxylated glycerol. Cremophor is used as an emulsifying agent and aqueous solubilizer for hydrophobic products including certain drugs, fat-soluble vitamins, animal feed, and cosmetics. It is present in the i.v. formulations of the anticancer drugs teniposide and paclitaxel. The paclitaxel formulation (Taxol; Bristol Myers Squibb Co, Wallingford, CT) consists of 6 mg/ml in 50% ethanol and 50% cremophor. Thus, a patient receiving paclitaxel at a widely used dose of 175 mg/m² will also receive ~14 ml/m² of cremophor.

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1 To whom requests for reprints should be addressed, at Sydney Cancer Centre, Royal Prince Alfred Hospital, Sydney 2050, Australia. Phone: 61-2-95157680; Fax: 61-2-95191546; E-mail: michaelm@cancer.nsw.gov.au.

2 The abbreviations used are: MDR, multidrug resistance; P-gp, P-glycoprotein; cremophor, Cremophor EL; AUC, area under the concentration versus time curve; CV, coefficient of variation.
While examining the MDR-reversing potential of compounds dissolved with cremophor, we observed that cremophor itself was able to reverse MDR in tumor cells in vitro (20, 21). Other groups have reported similar results (22, 23). The effect of cremophor on increasing the sensitivity of MDR cells is rapid and reversible and may be either due to a direct interaction with P-gp (22, 24) or the result of a general membrane perturbation affecting the function of this protein pump (25, 26). Cremophor concentrations \( \geq 0.1 \, \mu\text{g} / \text{ml} \) produce some increase in the sensitivity of MDR cells, with –50% reversal at 1.0 \( \mu\text{g} / \text{ml} \) and complete reversal to wild-type sensitivity occurring at concentrations of 1.5–2.0 \( \mu\text{g} / \text{ml} \) (27, 28).

In patients receiving paclitaxel formulated with cremophor, we have measured the resulting plasma cremophor concentrations using a bioassay. This assay depends on the ability of cremophor to alter the intracellular daunorubicin fluorescence in the MDR-expressing human T-cell leukemia cell line CEM/VLB100. The cremophor levels in patients after short infusions of 175 mg/m² paclitaxel (0.8–1.8 \( \mu\text{g} / \text{ml} \)) are in the range in which MDR modulation occurs in vitro (27, 29).

Cremophor can cause histamine release in dogs (30) and has been implicated in the hypersensitivity reactions observed in patients receiving paclitaxel (31). Such reactions can be successfully prevented in nearly all patients by the use of a prolonged infusion of paclitaxel and the administration of prophylactic antiallergy premedication (31). Other than hypersensitivity reactions, no clear toxicity to humans has been described from cremophor. It is therefore suitable for clinical investigation as a potential MDR modulator.

The aim of this clinical trial was to investigate the use of cremophor as a potential MDR modulator by determining: (a) the feasibility of administering 6-h infusions of cremophor with bolus doxorubicin; (b) whether plasma levels of cremophor sufficient to modulate MDR in vitro could be obtained without limiting toxicity; (c) the pharmacokinetics of cremophor at different doses after a 6-h infusion; and (d) the effect of cremophor on the pharmacokinetics of doxorubicin and its principal metabolite, doxorubicinol.

**PATIENTS AND METHODS**

**Eligibility.** Patients were required to have histologically proven advanced cancer resistant to conventional chemotherapy or for which no satisfactory chemotherapy exists. Other eligibility criteria were: performance status 0–2 by the criteria of the Eastern Cooperative Oncology Group and life expectancy >8 weeks, absolute neutrophil count \( \geq 2.0 \times 10^9 / \text{liter} \), platelet count \( \geq 150 \times 10^9 / \text{liter} \), serum bilirubin <1.5 times upper limit of normal for the institution, serum creatinine \( \leq 120 \, \mu\text{mol} / \text{liter} \), and cardiac ejection fraction \( \geq 50\% \) determined by radionuclide angiography imaging. Patients with serious concurrent medical illnesses or requiring concurrent treatment with calcium channel blocking agents (for example verapamil, nifedipine, and diltiazem) were excluded. Written informed consent was obtained from all patients, and the protocol was approved by the Institutional Ethics Committee of the Peter MacCallum Cancer Institute. Separate written informed consent was obtained for participation in the pharmacokinetic studies.

**Trial Design.** This trial was performed in three stages. In the first stage (dose levels 1–4, Table 1) all patients received 50 mg/m² doxorubicin as a bolus, and the dose of cremophor was escalated in cohorts of three to seven patients. When end-infusion cremophor concentrations were measured to be \( \geq 1.0 \, \mu\text{g} / \text{ml} \), a cross-over design was used to investigate the influence of this dose of cremophor on doxorubicin pharmacokinetics. This cohort of patients (dose level 4A, Table 1) were randomized to receive either 50 mg/m² doxorubicin with cremophor or the same dose of doxorubicin alone in the first cycle and the alternative treatment in the second cycle. In the third stage (dose levels 5 and 6, Table 1), the dose of cremophor was further escalated, and patients were randomized to receive either doxorubicin with cremophor or 50 mg/m² doxorubicin in the first cycle and the alternative treatment in the second cycle. The dose of doxorubicin given with cremophor to these cohorts was calculated to produce the same doxorubicin plasma AUC as 50 mg/m² doxorubicin when given alone.

The initial dose level was selected as a minimum cremophor exposure because of the possible risk of hypersensitivity. Subsequent dose escalation was based on the calculated exposure of patients receiving paclitaxel to cremophor and the levels obtained (27, 29) and the results obtained from lower dose levels in this trial.

At all dose levels, cycles were repeated every 3 weeks. The cremophor dose was not escalated for any individual patient. No maximum number of cycles was specified. Patients participating in the cross-over stages of the trial received additional treatment with doxorubicin and cremophor after the second cycle. Prophylactic use of recombinant colony-stimulating factors to prevent myelosuppression was not permitted.

While on study, complete blood count including differential, electrolytes, and liver function tests were performed three times a week. Patients were reviewed weekly for symptoms and signs of toxicity for at least the first two cycles. Tumor imaging was repeated after every two cycles. Toxicity and response to treatment were graded by the standard criteria of the WHO (32). Limiting toxicity was defined as febrile neutropenia, grade IV thrombocytopenia or anemia, or grade III or IV nonhematological toxicity (excluding nausea, vomiting, and alopecia). Uncomplicated grade IV neutropenia was not considered a limiting toxicity.

**Drug Administration.** Cremophor was obtained from BASF Chemicals and formulated for this trial as a sterile 100-ml solution containing 25% v/v cremophor by F. H. Faulding Pharmaceuticals (Mulgrave, Australia). This was further dissolved in 1 liter normal saline solution and infused as a 6-h i.v.
infusion. Patients received a standard premedication consisting of 8 mg dexamethasone, 50 mg ranitidine, and 25 mg promethazine, all given i.v. 30 min before the commencement of cremophor. As a further precaution, in the first stage of the trial, patients received the first 50 ml of the cremophor infusion over 30 min with the remainder over 5.5 h. Because no hypersensitivity reactions were observed, this precaution was dropped for subsequent patients.

All patients received doxorubicin as an i.v. bolus. Doxorubicin was given 1 h after the commencement of the cremophor infusion. For the cross-over pharmacokinetic part of the trial, on the doxorubicin-alone cycle, patients also received a control infusion of 1 liter of normal saline commencing 1 h before the administration of doxorubicin. Additionally, the same premedication was given 30 min before the control infusion to eliminate the effect of any pharmacokinetic interaction between doxorubicin and these drugs.

**Pharmacokinetics.** Cremophor was measured in plasma during and after the patient’s first infusion of cremophor. Initially, cremophor levels were measured in patients at dose levels 3 and 4 at three time points, mid-infusion, end-infusion, and 1 h after infusion. At subsequent dose levels, more detailed sampling was performed to define the pharmacokinetics of cremophor. Samples were collected at the following times: prior to the administration of premedication; immediately after the administration of doxorubicin; 2, 3, and 5 h after the start of the cremophor infusion; end-infusion; and 1, 3, 7, 19, 31, and 43 h after the completion of the cremophor infusion. Blood (10 ml) was collected into heparinized tubes and centrifuged immediately, and the plasma was stored at −70°C.

Cremophor was measured using a bioassay as described previously (27, 29). Briefly, this assay depends on the ability of cremophor in the plasma sample to modulate the equilibrium intracellular fluorescence of daunorubicin assessed by flow cytometry in the MDR-expressing cell line CEM/VLB1oo. The patient’s pretreatment plasma was used to derive a six-point standard curve for that patient against which the subsequent samples were compared. All measurements were done in triplicate. The within-day CV was <10%, the interassay CV was <14%, and the lower limit of detection was 0.05 μl/ml. The assay could not reliably quantify cremophor levels of >2.0 μl/ml, even with dilution; consequently, samples measured to be >2.0 μl/ml were analyzed as being equal to 2.0 μl/ml.

Doxorubicin and doxorubicinol were measured in plasma from patients at dose levels 4A, 5, and 6 after doxorubicin alone and the first course of doxorubicin given with cremophor. Samples were collected at the following times: prior to the administration of doxorubicin; immediately after the administration of doxorubicin; and 10, 20, and 40 min and 1, 2, 4, 6, 8, 12, 24, 36, and 48 h after doxorubicin. Blood (10 ml) was collected into heparinized tubes and centrifuged immediately, and the plasma was stored at −70°C. When the sample times for cremophor and doxorubicin coincided, a 20-ml blood sample was divided and prepared separately for the assays.

Doxorubicin and doxorubicinol were measured in plasma by an HPLC method modified from Maessen et al. (33) using daunorubicin as the internal standard. Samples were prepared by solid phase extraction of 1.0 ml of plasma using C18 Sep-Pak Vac 1-ml cartridges (Waters, Milford, CT) with the solvent volumes reduced to match the amount of sorbent. Separation was on a C18 Nova-Pak Radial-Pak cartridge (8 mm × 10 cm) with a Nova-Pak C18 Guard-Pak (both Waters) using a gradient mobile phase (A: 20% acetoniitrile, 0.02 m NaH2PO4, pH 4; B: 45% acetoniitrile, 0.02 m NaH2PO4, pH 4). Samples were quantified by fluorescence detection (excitation wavelength, 480 nm; emission wavelength: 580 nm) against a log-weighted linear standard curve in human plasma from 0.5 to 3125 ng/ml for both doxorubicin and doxorubicinol. The minimum quantifiable concentration (lowest concentration with a CV <20%) was 0.5 ng/ml.

Pharmacokinetic parameters were calculated using the SIFPAR program (SIMED, Creteil, France). For cremophor, model-independent analyses were performed. The terminal elimination half-life was determined by linear regression of the final four points on the log-linear concentration-time profile. The AUC for cremophor was calculated using the trapezoidal method and extrapolated to infinity (AUCinf). Total clearance of cremophor was calculated as dose/AUCinf normalized to body surface area. Because the bioassay measures a pharmacological effect of cremophor rather than a defined chemical compound, the terms “apparent AUC” and “apparent clearance” are used for cremophor.

For doxorubicin, pharmacokinetic parameters were estimated by nonlinear weighted least-squares analysis using the Powell minimization algorithm. Individual models were chosen on the basis of Akaike’s Information Criterion and visual inspection of the observed versus fitted concentration-time points. The parameters computed from the model were: half-lives, AUCinf, volume at steady-state (VSS), mean residence time (MRT), and clearance (CL). Additionally, a model-independent analysis calculated the doxorubicin AUC to the last measured time point (AUCt) by the trapezoidal method. The doxorubicinol AUCt was calculated by the same method as the doxorubicin AUCt, and the metabolite ratio was used to describe the exposure to the metabolite relative to the parent drug.

**Statistics.** Pharmacokinetic parameters for doxorubicin and doxorubicinol and neutropenia in the presence and absence of cremophor were compared using the Wilcoxon signed rank test. Cremophor pharmacokinetics at different doses were compared using the Mann Whitney test. All Ps are two-sided, and those <0.05 were considered significant.

**RESULTS**

Forty-one patients were enrolled on this trial (Table 2). Most patients had received prior chemotherapy with 20 of 41 (50%) having had at least one chemotherapy drug capable of inducing P-gp-associated MDR. Of these patients, 12 had previously received an anthracycline (doxorubicin or epirubicin), 8 had received etoposide, 7 had received a Vinca alkaloid, and 3 had received paclitaxel. The number of patients enrolled at each dose level is given in Table 1. One patient entered at dose level 4A received doxorubicin alone in the first cycle but developed rapid symptomatic deterioration and was removed from the study, having never received doxorubicin with cremophor. Two patients entered at dose level 4A, two patients at dose level 5, and one patient at dose level 6 received only doxorubicin plus cremophor because they went off study after the first cycle or
Thrombocytopenia was rare, with two patients having grade IV evaluated because the doxorubicin dose was reduced after he-because of a lack of nadir counts. Another eight cycles were not combination of doxorubicin and cremophor following 74 cycles because of unwillingness to participate in the pharmacokinetic studies.

**Toxicity.** Hematological toxicity was evaluated with the combination of doxorubicin and cremophor following 74 cycles in 39 patients. One patient at level 4A could not be evaluated because of a lack of nadir counts. Another eight cycles were not evaluated because the doxorubicin dose was reduced after hematological toxicity in previous cycles.

The principal hematological toxicity was neutropenia. The incidence of grade IV neutropenia increased with increasing cremophor doses. Grade IV neutropenia occurred in 0 of 3 patients at level 1, 1 of 4 patients at level 2, 4 of 6 patients at level 3, and 11 of 16 patients at levels 4 and 4A. With the reduction of doxorubicin dose, grade IV neutropenia occurred in 1 of 6 patients at level 5 and 2 of 4 patients at level 6. Febrile neutropenia occurred in one patient at level 3, three patients at levels 4 and 4A, one patient at level 5, and one patient at level 6. There was one death at level 4 from neutropenic sepsis. Thrombocytopenia was rare, with two patients having grade IV thrombocytopenia (one at level 4A and one at level 6), both in the setting of febrile neutropenia. One patient at level 4A had grade IV anemia.

In six patients at level 4A, neutropenia could be evaluated in the first two cycles in the presence and absence of cremophor. All these patients received 50 mg/m² doxorubicin. The mean neutrophil nadir was 1.9 × 10⁹/liter after doxorubicin alone and 0.58 × 10⁹/liter after doxorubicin with cremophor (P = 0.03; Fig. 1a). Three patients at level 5 and three patients at level 6 had neutropenia evaluated in the first two cycles. These patients received 50 mg/m² doxorubicin alone and 35 mg/m² with cremophor. The mean neutrophil nadir was 1.7 × 10⁹/liter after doxorubicin alone and 1.4 × 10⁹/liter after doxorubicin with cremophor (P = 0.16; Fig. 1b).

Other doxorubicin-related toxicity was infrequent. Stomatitis was recorded in 12 patients, but only 2 reached grade III (one patient at level 4A and one at level 5). Only three patients received five or more cycles of doxorubicin with cremophor. One patient at level 4 developed angina after eight cycles. This patient had previously documented ischemic heart disease and had undergone a coronary angioplasty. His pre-study left ventricular ejection fraction was 61%; after six cycles of doxorubicin and 30 ml/m² cremophor, it was 56%, and after eight cycles, it had fallen to 43% with global dysfunction, indicating anthracycline cardiotoxicity. After an additional angioplasty, the angina resolved, but the ejection fraction did not improve. One patient at dose level 1 received nine cycles of doxorubicin and cremophor and one patient at level 5 received five cycles of doxorubicin and cremophor plus one cycle of doxorubicin alone. Neither of these patients had any fall in their ejection fraction.

No major hypersensitivity reactions to cremophor were observed, and no patients had their infusion discontinued or modified. Toxicities considered potentially related to cremophor were cutaneous (pruritus, flushing, or rashes), hypotension or dizziness, and headache. Because of the subjective nature of some of these symptoms, they were graded as grade I (mild and not requiring treatment or interfering with function), grade II

<table>
<thead>
<tr>
<th>Table 2 Patient details (n = 41)</th>
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<tbody>
<tr>
<td>Age</td>
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<td>Range</td>
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<tr>
<td>Sex</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>Tumor types</td>
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<tr>
<td>Colorectal</td>
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<tr>
<td>Renal</td>
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<tr>
<td>Melanoma</td>
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<tr>
<td>Sarcoma</td>
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<tr>
<td>Breast</td>
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<tr>
<td>Unknown primary</td>
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<tr>
<td>Non-small cell lung</td>
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<tr>
<td>Hepatoma</td>
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<tr>
<td>Gastric</td>
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<tr>
<td>Ovary</td>
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<tr>
<td>Non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>Small round cell/Ewing's</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>Prior chemotherapy</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes, non-MDR drugs</td>
</tr>
<tr>
<td>Yes, MDR drugs</td>
</tr>
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</table>

![Fig. 1 Nadir absolute neutrophil count (ANC) in patients receiving either doxorubicin (Dox) alone or doxorubicin with cremophor. Each pair of points represents an individual patient. a. patients receiving 50 mg/m² doxorubicin alone or the same dose with 30 ml/m² cremophor. b. patients receiving 50 mg/m² doxorubicin alone or 35 mg/m² doxorubicin with cremophor 45 ml/m² (—) or 60 ml/m² (—).](image_url)
(moderate causing some impairment of function but not requiring hospitalization), or grade III (severe requiring hospitalization or causing significant interference with function). No grade II or III cremophor toxicity was recorded at the first three dose levels. One patient at level 4A had grade II dizziness, one patient at level 5 had grade II rash, one patient at level 5 had grade III headache and grade III pruritus, and one patient at level 6 had grade III hypotension and grade III rash. In none of these patients did these symptoms occur when the patient received doxorubicin alone, confirming their relationship to cremophor. These symptoms commenced several hours to days after cremophor and persisted for 1–2 weeks with the exception of the patient at level 5, whose pruritus gradually resolved over 3 months after discontinuing cremophor.

Transient increases in bilirubin were recorded in two patients at level 3, one patient at level 4, and one patient at level 4A. All of these were grade II. One patient at level 4 had grade III hyperbilirubinemia in the setting of severe sepsis. One patient at level 6 had grade III hyperbilirubinemia after both doxorubicin with cremophor and doxorubicin alone. Another patient at level 6 developed jaundice 3 weeks after doxorubicin and cremophor, but investigations showed progressive hepatic metastases.

Limiting toxicity did not occur at the first two levels. At level 3, limiting toxicity occurred in 1 of 6 patients (17%), at level 4 in 5 of 17 (29%), at level 5 in 2 of 6 (33%), and at level 6 in 2 of 4 (50%).

Response. Responses were observed in two patients. One patient at dose level 4 with hepatoma that had not responded to previous epirubicin had multifocal hepatic lesions on computed tomography scan with a serum α-fetoprotein of 15,700 units/liter at baseline. Over eight cycles of doxorubicin with cremophor, the α-fetoprotein fell to 23 units/liter (normal, <20 units/liter) with minor residual abnormalities on computed tomography. Treatment was discontinued because of potential cardiac toxicity, and the response duration was 56 weeks. One patient at level 4A with a small round cell tumor who had received two prior chemotherapy regimens including doxorubicin had a 48% reduction in the size of intraabdominal lymph nodes lasting 16 weeks.

Cremophor Pharmacokinetics. Plasma cremophor concentrations were measured in three patients at level 3 and four patients at level 4. None of the patients at level 3 reached a cremophor concentration of 1.0 μl/ml, with mid-infusion concentrations ranging from 0.30–0.76 μl/ml and end-infusion concentrations ranging from 0.36–0.92 μl/ml. With 30 ml/m² cremophor (level 4), all four patients tested had a plasma cremophor concentration >1.0 μl/ml, with mid-infusion concentrations ranging from 1.06–1.48 μl/ml and end-infusion concentrations ranging from 0.96–1.16 μl/ml. Two patients had concentrations ≥1.0 μl/ml at 1-h after infusion. Because 1.0 μl/ml cremophor produces some reversal of MDR in vitro (27, 28), a more detailed study of cremophor levels was done at subsequent dose levels.

Cremophor pharmacokinetics at these dose levels are presented in Table 3. There were less than proportionate increases in maximal concentrations and apparent AUCinf with increasing doses. The apparent clearance of cremophor was significantly faster after 60 ml/m² than after 30 ml/m² (P = 0.02). All patients at level 4A achieved a plasma cremophor concentration >1.0 μl/ml, but only two reached 1.5 μl/ml. All patients at level 5 achieved 1.5 μl/ml, with 2 reaching ≥2.0 μl/ml. At level 6, only two patients achieved 1.5 μl/ml, with one reaching ≥2.0 μl/ml.

**Table 3. Cremophor pharmacokinetics**

<table>
<thead>
<tr>
<th>Level</th>
<th>Cremophor dose (ml)</th>
<th>CMAX (μl/ml) Mean ± SD</th>
<th>CDOX (μl/ml) Mean ± SD</th>
<th>Apparent AUCinf (μl/ml·h) Mean ± SD</th>
<th>Apparent CI (ml/h/m²) Mean ± SD</th>
<th>Half-life (h) Mean ± SD</th>
</tr>
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<tr>
<td>4A</td>
<td>30 ml/m²</td>
<td>54.7 ± 6.8</td>
<td>1.33 ± 0.17</td>
<td>0.87 ± 0.18</td>
<td>28.0 ± 5.8</td>
<td>1124 ± 303</td>
</tr>
<tr>
<td>5</td>
<td>45 ml/m²</td>
<td>80.6 ± 9.3</td>
<td>1.85 ± 0.17</td>
<td>1.34 ± 0.42</td>
<td>33.4 ± 13.4</td>
<td>1543 ± 610</td>
</tr>
<tr>
<td>6</td>
<td>60 ml/m²</td>
<td>106.9 ± 4.3</td>
<td>1.63 ± 0.32</td>
<td>1.41 ± 0.46</td>
<td>31.9 ± 15.6</td>
<td>2205 ± 944</td>
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*CMAX, maximum measured cremophor concentration; CDOX, cremophor concentration at time of bolus doxorubicin administration; CI, clearance.*

Pharmacokinetics of Doxorubicin and Doxorubicinol. Pharmacokinetics of doxorubicin and doxorubicinol in the presence and absence of 30 ml/m² cremophor were evaluated in eight patients in the cross-over design at level 4A. The pharmacokinetic parameters are listed in Table 4, and representative patients are shown in Fig. 2. The doxorubicin clearance and AUCinf were significantly altered in the presence of cremophor (P = 0.02). The increase in doxorubicin AUCinf was 28% ± 27% (mean ± SD) with a range of −2% to 79%. The doxorubicinol AUC inf was also significantly increased in the presence of cremophor (P = 0.02), and the magnitude of the increase was greater at 105% ± 61% (mean ± SD). This resulted in the metabolite ratio being significantly higher in the presence of cremophor (P = 0.008).

At dose levels 5 and 6, the doxorubicin dose was reduced by 30% to balance the increase in AUCinf that occurred in the presence of 30 ml/m² cremophor. Three patients at level 5 and three patients at level 6 had doxorubicin and doxorubicinol pharmacokinetics studied in the cross-over design. Results from these six patients were combined because there were no statistically significant differences between the two groups. The doxorubicin AUCinf (mean ± SD) was 1655 ± 439 ng/ml·h after 50 mg/m² doxorubicin and 1666 ± 280 ng/ml·h after 35 mg/m² doxorubicin with cremophor (P = 0.6). As expected, the doxorubicin clearance was slower in the presence of cremophor, being 530 ± 110 ml/min/m² (mean ± SD) for doxorubicin without cremophor and 364 ± 64 ml/min/m² (mean ± SD) for doxorubicin in the presence of cremophor (P = 0.03). Despite the lower doxorubicin dose, the AUC for doxorubicinol was higher after 35 mg/m² doxorubicin with cremophor than after 50
Table 4  Pharmacokinetics (mean ± SD) of doxorubicin and doxorubicinol

<table>
<thead>
<tr>
<th>Dose level 4</th>
<th>50 mg/m² doxorubicin</th>
<th>50 mg/m² doxorubicin with cremophor</th>
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<tbody>
<tr>
<td>Doxorubicin</td>
<td>AUC$_{24h}$ (ng/ml · h) 1448 ± 350</td>
<td>1786 ± 264⁴</td>
</tr>
<tr>
<td></td>
<td>CI (ml/min/m²) 612 ± 179</td>
<td>477 ± 70⁴</td>
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<td></td>
<td>MRT (h) 17.0 ± 8.0</td>
<td>21.0 ± 5.1</td>
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<td></td>
<td>V_{ss} (l/m²) 618 ± 311</td>
<td>567 ± 116</td>
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<tr>
<td></td>
<td>T_{1/2a} (h) 0.08 ± 0.02</td>
<td>0.10 ± 0.02</td>
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<tr>
<td></td>
<td>T_{1/2b} (h) 1.7 ± 1.3</td>
<td>2.9 ± 1.3</td>
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<tr>
<td></td>
<td>T_{1/2c} (h) 22.5 ± 8.4</td>
<td>25.7 ± 5.9</td>
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<tr>
<td>Doxorubicinol</td>
<td>AUC$_{144h}$ (ng/ml · h) 252 ± 104</td>
<td>486 ± 107⁹</td>
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<td>Metabolite ratio 0.23 ± 0.07</td>
<td>0.37 ± 0.11⁹</td>
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<th>Dose levels 5 and 6</th>
<th>50 mg/m² doxorubicin</th>
<th>35 mg/m² doxorubicin with cremophor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>AUC$_{24h}$ (ng/ml · h) 1655 ± 439</td>
<td>1666 ± 280</td>
</tr>
<tr>
<td></td>
<td>CI (ml/min/m²) 530 ± 110</td>
<td>364 ± 64⁴</td>
</tr>
<tr>
<td>Doxorubicinol</td>
<td>AUC$_{144h}$ (ng/ml · h) 453 ± 272</td>
<td>860 ± 428⁸</td>
</tr>
<tr>
<td></td>
<td>Metabolite ratio 0.31 ± 0.11</td>
<td>0.60 ± 0.22⁹</td>
</tr>
</tbody>
</table>

⁴ P = 0.02.
⁵ P = 0.008.
⁹ P = 0.03.

mg/m² doxorubicin alone (860 ± 428 ng/ml-h versus 453 ± 272 ng/ml-h; P = 0.03). The metabolite ratio was 0.31 ± 0.11 after 50 mg/m² doxorubicin and 0.60 ± 0.22 after 35 mg/m² doxorubicin with cremophor (P = 0.03).

DISCUSSION

In the initial clinical evaluation of compounds as potential modulators of MDR, it is important to establish that adequate amounts of the drug can be administered without producing excessive normal tissue toxicity. The type of assay used in this trial allows a more direct measurement of the desired biological effect of the modulator than measurement of total or free plasma drug levels and has been used by several groups investigating new modulators such as PSC 833, S9788, and dexverapamil (15, 34, 35). Although issues, including the standardization of such assays, and the relationship between plasma and tumor bioassay results require further investigation, a bioassay was particularly appropriate for evaluating cremophor, because when this trial was performed, the specific component of cremophor that produces MDR reversal had not been identified, and consequently no specific assay for it was possible.

When given as a 6-h infusion, 45 ml/m² cremophor resulted in plasma levels sufficient to potentially reverse P-gp-mediated MDR in all patients. Although the limitations of the bioassay do not allow definitive conclusions about the possible nonlinear pharmacokinetics of the MDR-modulating component(s) of cremophor to be drawn, the 45 ml/m² dose appears most suitable for testing cremophor as a potential MDR modulator in Phase II trials.

Using the same assay, we have reported previously that in the same patient population after 175 mg/m² paclitaxel over 3 or 6 h, the apparent half-life of cremophor was 26.1 ± 8.8 h and 26.4 ± 8.0 h, respectively (29). This is longer than found in this Phase I study. It is possible that at the lower doses corresponding to this paclitaxel dose, cremophor has a different pharmacokinetic behavior. It may also reflect that in the previous study (29), cremophor levels were only measured up to a maximum of 24 h after completion of the paclitaxel infusion, whereas in this study, more protracted sampling was undertaken. The present study is therefore likely to have more accurate calculations of the apparent half-life. It is also possible that when given combined with paclitaxel and ethanol, cremophor pharmacokinetics are different than when cremophor is given alone.

In this trial, all patients were given a standard premedication schedule. No acute hypersensitivity reactions were observed during cremophor infusions, but toxicities such as rash, pruritus, and hypotension seen at the higher cremophor doses are likely to be drug related. The cross-over design allows the elimination of doxorubicin or the premedications themselves as causative factors. It is possible that a more protracted regimen of corticosteroids and/or antihistamines could reduce or prevent these symptoms. One patient had persistent pruritus after cre-
mophor, a toxicity reported recently in 5 of 14 patients who received high doses of paclitaxel (250–265 mg/m² over 3 h; Ref. 36).

Although antitumor activity was not an end point of this Phase I trial, two responses were observed in pretreated patients, including an almost complete response in a patient with hepatoma previously resistant to anthracycline. Tumor biopsy for P-gp expression was not required in this trial, but hepatomas are known to express high levels of P-gp (37). This impressive response demonstrates that additional studies of potential MDR modulators such as cremophor in patients with hepatoma are indicated.

A significant pharmacokinetic interaction between cremophor and doxorubicin was demonstrated in this trial. When administered with cremophor, the doxorubicin AUC increased by ~30%. There was an even greater increase in the AUC of doxorubicin. We have shown previously that in mice, cremophor produced a similar pharmacokinetic interaction with doxorubicin and doxorubicinol (38). This successful prediction shows the potential of such preclinical studies to help design clinical trials with potential MDR modulators. The pharmacodynamic end point of neutropenia was measured and found to be significantly greater when doxorubicin was given with cremophor.

The reason for this pharmacokinetic interaction could not be determined from this trial. Doxorubicin is metabolized to doxorubicinol by cytoplasmic aldoketoreductase enzymes, and doxorubicinol is further metabolized to noncytotoxic aglycones by microsomal P450 reductase. A similar effect to that observed in this trial with cremophor has been reported when the MDR modulator cyclosporin A was combined with doxorubicin (13). Thus with cyclosporin A the doxorubicin AUC increased by 55%, and the doxorubicinol AUC increased by 350% with increased neutropenia (13). In contrast, another trial showed increased doxorubicin AUC but a lower then expected metabolite ratio (14) when doxorubicin and cyclosporin A were combined; however, patients were not evaluated after doxorubicin alone (14). Pharmacokinetic interactions have also been reported when doxorubicin has been combined with other MDR modulators (39, 40), as well as when other drugs primarily metabolized in the liver such as etoposide and paclitaxel are combined with MDR modulators (15, 41, 42). Generally, such interactions are characterized by slower clearance of the cytotoxic agent and increased normal tissue toxicity. Direct inhibition of P-gp in liver, biliary tract, and kidney, inhibition of metabolizing enzymes such as hepatic cytochrome P450 systems, and alterations of protein binding of the cytotoxic agent may occur as a result of combining the agent with an MDR modulator. It is likely that the observed pharmacokinetic interactions reflect a combination of these effects and may be dependent on the modulator and cytotoxic used and perhaps on the dose and schedule of the cytotoxic agent.

The pharmacokinetic results found in this trial are of relevance for the observed pharmacokinetic interaction between paclitaxel and doxorubicin. When paclitaxel was given as a 24-h infusion immediately after a 48-h infusion of doxorubicin, the doxorubicin clearance was reduced by 30% (43). Gianni et al. (44) recently reported a series of pharmacokinetic studies of the paclitaxel/doxorubicin doublet. When a 3-h infusion of paclitaxel was given immediately after bolus doxorubicin, the doxorubicin AUC increased by up to 30%, and the doxorubicinol AUC increased by up to 100% compared to when paclitaxel was commenced 24 h after doxorubicin. At least for doxorubicinol, the effect was more pronounced when the paclitaxel dose was increased from 150 to 200 mg/m². Concurrent 3-h administration of doxorubicin and paclitaxel led to lower doxorubicin and doxorubicinol AUCs than bolus doxorubicin followed by a 3-h paclitaxel infusion (44). In another study where both drugs were given by 72-h infusion, concurrent paclitaxel increased the steady-state concentration of doxorubicin and doxorubicinol, although only the latter was statistically significant (45). Overall, despite the different schedules of both drugs used, administration of paclitaxel reduces the clearance of doxorubicin and to a greater extent doxorubicinol. Our results indicate this is at least in part due to the cremophor in the paclitaxel formulation.

Because cremophor is not a pure substance, it is difficult to develop as a pharmaceutical. Studies to identify the specific component or components responsible for in vitro MDR reversal (46, 47) and develop potentially more active analogues (48) have shown some success. Additional clinical studies with cremophor and subsequently with these agents are indicated to develop this novel class of compounds. Other observed preclinical effects of cremophor may also translate into worthwhile clinical outcome (49).

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