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Inhibition of etoposide elimination in the isolated perfused rat liver by Cremophor EL and Tween 80

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Abstract Cremophor EL, a surfactant used in the clinical formulation of cyclosporine and paclitaxel, will reverse the multidrug resistance (MDR) phenotype in vitro. As other MDR modulators can alter the pharmacokinetics of cytotoxic drugs, the aim of this study was to examine the effect of Cremophor and another MDR-reversing surfactant, Tween 80, on the hepatic elimination and biliary excretion of etoposide. Using the isolated perfused rat-liver model with 80 ml recirculating perfusate containing 20% red blood cells and 4% bovine serum albumin, etoposide (1.6 mg) with and without Cremophor (800 or 80 mg) or Tween 80 (80 mg) was given into the perfusate reservoir, and perfusate and bile samples were collected for 3 h. Etoposide was measured by high-performance liquid chromatography (HPLC) and Cremophor was measured using a bioassay. Both surfactants changed the etoposide elimination profile from biphasic to monophasic. High-dose Cremophor increased the AUC (from 334 ± 23 to 1540 ± 490 μg min ml^{-1}, $P < 0.05$) and decreased the total clearance (from 4.8 ± 0.3 to 1.1 ± 0.3 ml/min, $P < 0.05$) and biliary clearance (from 2.6 ± 1.1 to 0.5 ± 0.2 ml/min, $P < 0.05$) but decreased the elimination half-life (from 62 ± 17 to 40 ± 5 min, $P < 0.05$) and volume of distribution (from 424 ± 85 to 65 ± 19 ml, $P < 0.05$). Low-dose Cremophor and Tween 80 caused intermediate effects on these parameters that were statistically significant for total clearance, half-life, and volume of distribution. Cremophor had no adverse effect on liver function, whereas Tween 80 caused haemolysis and cholestasis. The initial high-dose Cremophor perfusate concentration was 0.8 mg/ml, which previous studies have shown to be clinically relevant and close to the optimal level for MDR reversal in vitro (1.0 mg/ml). Cremophor may be a clinically useful MDR modulator, but it may alter the pharmacokinetics of the cytotoxic drug.

Key words Etoposide · Multidrug resistance · Chemosensitizers

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Introduction

One of the major reasons for failure of cancer chemotherapy is the presence of intrinsic or acquired drug resistance in malignant cells. Although a number of different mechanisms of drug resistance have been described, one of the most widely studied is multidrug resistance (MDR). Cells that display the MDR phenotype are cross-resistant to a broad spectrum of structurally and functionally unrelated drugs, including epipodophyllotoxins, vinca alkaloids, paclitaxel, and anthracyclines. Classic MDR is characterized by reduced intracellular accumulation of the cytotoxic drug due to overexpression of an energy-dependent plasma membrane transport protein, known as P-glycoprotein, which acts as an efflux pump for certain endogenous compounds and xenobiotics [4]. P-glycoprotein has been demonstrated in the cell membranes of many human tumours as well as normal tissues such as the adrenal, liver and small intestine as well as endothelial cells and haemopoietic stem cells [30].

Many compounds are capable of reversing the MDR phenotype in vitro by inhibiting the function of P-glycoprotein. These MDR modulators, or chemosensitizers, share some common structural features and include certain calcium channel blockers, quinolines, steroids, antioestrogens, and cyclosporins. There have been many clinical trials in which a chemosensitizer has been combined with standard cancer chemotherapy regimens in an attempt to modulate MDR [21].

However, it has proved difficult to achieve plasma concentrations of the MDR modulator that are known to reverse MDR in vitro without dose-limiting side effects. In addition, several of the studies reported a pharmacokinetic interaction resulting in increased plasma levels of cytotoxic drugs, including doxorubicin and etoposide, with increased toxicity [2, 16, 19].

Cremophor EL (Cremophor), a polyethoxylated castor oil derivative commonly used as a drug-solubiliser, can reverse the MDR phenotype in cells in culture [10, 23, 34]. The new anticancer agent and P-glycoprotein substrate paclitaxel (Taxol) is formulated in 50% Cremophor and 50% ethanol, and patients treated with paclitaxel receive a significant volume of a potentially active MDR-reversing agent. We have previously reported that plasma levels of Cremophor measured in patients at the end of a 3-h infusion of 135 or 175 mg/m^2 paclitaxel were 1–2 mg/ml [32], concentrations that are sufficient to reverse MDR in vitro. The only toxicity that has been linked with Cremophor is hypersensitivity reactions, and all patients treated with paclitaxel routinely receive prophylactic anti-allergy medication. Thus, Cremophor is a relatively non-toxic compound that may prove useful as an MDR modulator.

It is important to determine the effect of MDR-reversing agents such as Cremophor on the pharmacokinetics of the cytotoxic drugs that will be coadministered. We used the isolated perfused rat liver model to examine the effect of Cremophor on the hepatic elimination and biliary excretion of etoposide, a member of the MDR group of drugs. We also studied the effect of another common vehicle for water-insoluble drugs, Tween 80, which is a more potent MDR modulator than Cremophor in vitro [10, 35].

Materials and methods

Chemicals

Etoposide as pure chemical was provided by Bristol-Myers Squibb (Melbourne, Australia). Cremophor EL was purchased from BASF Chemicals (Melbourne, Australia). Acetonitrile was of high-performance liquid chromatography (HPLC) grade (BDH Chemicals, Kilsyth, Vic., Australia); phenytoin, Tween 80 (polysorbate 80) and all other reagents were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, Mo., USA).

Liver perfusion

The experimental protocol was approved by the institutional Animal Experimentation Ethics Committee. Non-fasting male Sprague-Dawley rats (200–280 g) were anaesthetised with sodium pentobarbitone (60 mg/kg i.p.) and their livers were surgically isolated by standard techniques and perfused as previously described [33]. Briefly, the portal vein, thoracic inferior vena cava and bile duct were cannulated and the liver was connected to a recirculating perfusion circuit in a constant-temperature (37°C) cabinet. The perfusate was delivered by a peristaltic pump at 15 ml/min. The perfusate (80 ml) consisted of 20% (v/v) washed human red blood cells, 4% (w/v) bovine serum albumin (BSA, Fraction V; Commonwealth Serum Laboratories, Melbourne, Australia) and 0.1% (w/v) d-glucose in a modified Krebs Henseleit buffer (pH 7.4). Sodium taurocholate was infused into the perfusate reservoir at 30 μmol/h to maintain bile flow. Liver viability was confirmed by normal values for bile flow (greater than 0.5 ml/h), perfusion back pressure (less than 8.0 cm H_2O), oxygen consumption (2.0–4.0 μmol/g liver^{-1} min^{-1}) and homogeneous appearance. Livers remained viable for the duration of the 3-h experiment unless stated otherwise.

Drug administration and sampling

All drugs were given as a bolus dose into the perfusate reservoir to simulate systemic administration. Etoposide (1.6 mg) was dissolved in 20 μl dimethylsulfoxide (DMSO), and vehicle controls received 20 μl DMSO. In the relevant experiments, Cremophor or Tween 80 was added 5 min prior to etoposide. Tween 80 (80 mg) and Cremophor at a low dose (80 mg) or high dose (800 mg) were added to achieve nominal initial perfusate concentrations of either 1.0 or 10.0 mg/ml. Control experiments to detect effects on liver viability included: drug-free control ($n = 4$), low-dose Cremophor ($n = 3$), high-dose Cremophor ($n = 4$), Tween 80 ($n = 3$), and DMSO ($n = 5$). Experiments for comparison of etoposide pharmacokinetics included etoposide alone ($n = 4$), etoposide plus low-dose Cremophor ($n = 4$), etoposide plus high-dose Cremophor ($n = 5$), and etoposide plus Tween 80 ($n = 4$).

Samples for drug estimation were taken from the reservoir and were replaced with equal volumes of fresh perfusate. Drug amounts lost through sampling were less than 5% of the dose. Perfusate (750 μl) was sampled at 2, 5, 10, 20, 30, 60, 90, 120, 150 and 180 min after the addition of etoposide. Red blood cells were removed by centrifugation. Bile was collected in pre-weighed vials on ice at 30-min intervals and an aliquot (100 μl) was immediately diluted with 200 μl of 14 m*M* diammonium phosphate buffer (pH 5.2) as etoposide's stability is optimal in the pH range of 5.0–6.1. All samples were stored at -20°C prior to assay.

Drug assays

Etoposide was assayed in perfusate and bile using reverse-phase HPLC. Sample preparation involved solid-phase extraction of 500 μl perfusate or 200 μl bile with C_{18} (100-mg) cartridges (Vac Elute; Millipore Waters, Milford, Mass, USA). Etoposide and the internal standard phenytoin were eluted with 500 μl methanol and 20 μl was injected using an autosampler (WISP model 712). Separation was performed on a Waters Novapak phenyl stainless steel column (5 μm × 15 cm × 4.6 mm) with a Brownlee C_{18} (1.5-cm) guard column and a mobile phase of either 35% acetonitrile (perfusate) or 28% acetonitrile (bile) in 0.2 *M* sodium acetate buffer (pH 4.4) at a flow rate of 1.5 ml/min. Peaks were detected with a Spectra Physics Focus UV/VIS detector at 237 nm. Calibration was done against a linear standard curve of etoposide in drug-free human plasma (0.2–20.0 μg/ml for perfusate and 5.0–200 μg/ml for bile). The recovery at 10 μg/ml was 95%. The coefficient of variation (CV) for inter- and intra-assay precision at 0.4 and 16.0 μg/ml was less than 4%, and accuracy was within 4%. The minimal quantifiable concentration (lowest concentration measurable with a CV of $< 10\%$) was 0.1 μg/ml.

For confirmation that Cremophor remained present during the perfusion, perfusate (2.4 ml) was sampled at 3, 60, 120 and 180 min in selected control experiments, and Cremophor concentrations were determined using a bioassay in which intracellular daunorubicin fluorescence is measured in an MDR-expressing cell line (VLB_{100} cells, derived from CCRF-CEM human leukaemia) [32]. This

fluorescence increases when the P-glycoprotein is inhibited by reversing agents such as Cremophor. Perfusate levels of Cremophor were estimated by comparison with a standard curve generated by the addition of known amounts of Cremophor to drug-free perfusate.

Pharmacokinetics and statistics

Pharmacokinetic parameters were calculated using standard model-independent formulae. The area under the perfusate etoposide concentration versus time curve (AUC) was calculated by the trapezoidal method. The etoposide elimination half-life was estimated from the slope of the terminal points on the log-linear concentration-time curve. The volume of distribution was calculated as the total clearance times the elimination rate constant. The total perfusate clearance of etoposide was estimated as the dose divided by the perfusate $AUC_{(0-\infty)}$, and the biliary clearance was defined as the total amount excreted in bile divided by the perfusate $AUC_{(0-180\ min)}$. The biliary excretion rate constant of etoposide was calculated for each liver from the terminal phase slope of the semilogarithmic plot of the amount excreted in each 30-min interval versus the midpoint of that interval. Results were analysed by one-way analysis of variance followed by a Bonferroni multiple *t*-test. Statistical significance was defined as $P < 0.05$.

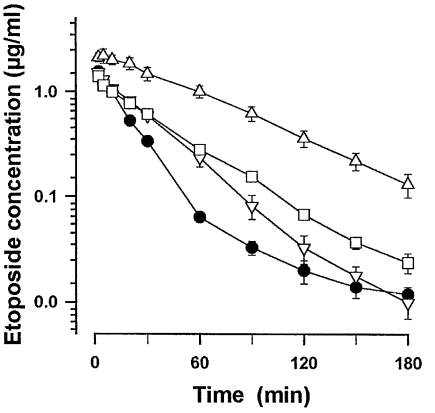

Fig. 1 Semilogarithmic plot of etoposide (*Etop*) perfusate elimination in the isolated perfused rat liver following a bolus dose of 1.6 mg etoposide alone (*black circles*) or in addition to a bolus dose of Cremophor (*crem*) at 800 (*triangles*) or 80 mg (*inverted triangles*) or Tween 80 at 80 mg (*squares*). Data represent mean values ± SEM, $n = 4$ or 5. Where error bars are not visible, the SEM is smaller than the size of the point

Results

The semilogarithmic plot of perfusate etoposide elimination from the isolated perfused rat liver was biphasic for etoposide alone but became monoexponential in the presence of Cremophor or Tween 80 (Fig. 1). High-dose Cremophor increased the AUC by nearly 5 orders of magnitude (Table 1), and there was a statistically significant dose-dependent decrease in total perfusate clearance. However, the terminal elimination half-life of etoposide decreased in the presence of Cremophor. In addition, although the etoposide volume of distribution was at least 5 times higher than the perfusate volume (424 ± 85 versus 80 ml perfusate volume), Cremophor significantly reduced this value. Tween 80 had effects similar to those of low-dose Cremophor on the pharmacokinetics of etoposide (Table 1).

Between 40% and 70% of the dose was excreted in bile as unchanged etoposide, with less than 2% being present in the liver or perfusate. The initial rate (0–30 min) of biliary etoposide excretion (19.2 ± 7.9 µg/min) was decreased by 68% following treatment with high-dose Cremophor (to 6.1 ± 1.7 µg/min) and by 45% following Tween 80 dosing (to 10.6 ± 2.5 µg/min; Fig. 2). After 60 min, the excretion rate was higher for all treatment groups as compared with the control, suggesting a recovery from the inhibitory effect. However, both high-dose Cremophor and Tween 80 decreased the biliary excretion rate constant (Table 1), and the decrease in biliary clearance accounted for approximately half of the decrease in total perfusate clearance. Nevertheless, the overall cumulative biliary excretion of etoposide was not significantly different among the groups (Table 1), again suggesting that the liver was capable of recovering from the exposure to surfactant.

Tween 80 caused a noticeable haemolysis that did not occur immediately following its addition but rather developed progressively over the 3-h period of the experiment, and the bile flow measured at 180 min was significantly lower than that determined in etoposide controls (42 ± 10 versus 70 ± 16 µl h^{-1} g $liver^{-1}$; Fig. 3). In contrast, Cremophor had no apparent

Table 1 Pharmacokinetic parameters for elimination of a 1.6-mg bolus dose of etoposide from the isolated perfused rat liver[a]

	n	Total clearance (ml/min)	$AUC_{0-\infty}$ (µg min ml^{-1})	Elimination half-life (min)	Volume of distribution (ml)	Biliary clearance (ml/min)	Biliary excretion rate constant ($min^{-1} \times 100$)	Cumulative biliary excretion (µg)
Control	4	4.8 ± 0.3	334 ± 23	62 ± 17	424 ± 85	2.6 ± 1.1	3.85 ± 0.40	816 ± 336
Cremophor (80 mg)	4	3.4 ± 0.5*	490 ± 85	29 ± 1*	139 ± 19*	2.3 ± 0.7	3.60 ± 0.39	1070 ± 198
Cremophor (800 mg)	5	1.1 ± 0.3*	1540 ± 490*	40 ± 5*	65 ± 19*	0.5 ± 0.2*	2.34 ± 0.61*	634 ± 180
Tween 80 (80 mg)	4	3.0 ± 0.2*	533 ± 34	35 ± 6*	143 ± 15*	1.4 ± 0.4	2.75 ± 0.33*	710 ± 192

* $P <$ 0.05 as compared with control values
[a] Data represent mean values ± SD

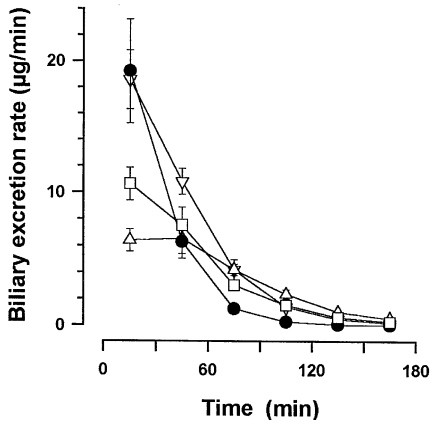

Fig. 2 Biliary excretion rate of etoposide (*Etop*) in the isolated perfused rat liver versus the midpoint of each 30-min bile collection following a bolus dose of 1.6 mg etoposide alone (*black circles*) or in addition to a bolus dose of Cremophor (*Crem*) at 800 (*triangles*) or 80 mg (*inverted triangles*) or Tween 80 at 80 mg (*squares*). Data represent mean values ± SEM, $n = 4$ or 5. Where error bars are not visible, the SEM is smaller than the size of the point

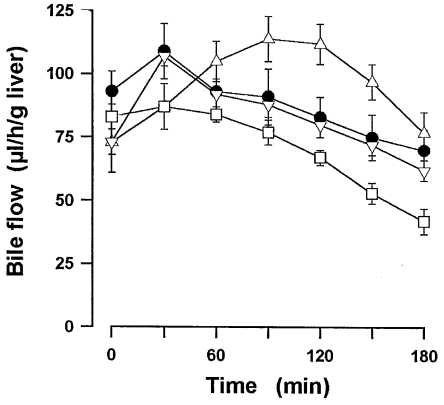

Fig. 3 Bile flow rate in the isolated perfused rat liver following a bolus dose of 1.6 mg etoposide (*Etop*) alone (*black circles*) or in addition to a bolus dose of Cremophor (*Crem*) at 800 (*triangles*) or 80 mg (*inverted triangles*) or Tween 80 at 80 mg (*squares*). Data represent mean values ± SEM, $n = 4$ or 5

adverse effect on liver viability over the duration of the experiments, although the bile flow attained with high-dose Cremophor was initially lower than that achieved with etoposide alone (not statistically significant) and then increased until 120 min, when it was higher (112 ± 18 versus 83 ± 17) $\mu l\ h^{-1}\ g\ liver^{-1}$, $P < 0.05$). In control experiments with Cremophor, Tween 80, or DMSO alone, the bile flow profiles were similar to those seen in the equivalent studies shown in Fig. 3, where etoposide was also present.

In some experiments, the concentration of Cremophor in perfusate was measured, and the doses of Cremophor added to the 80-ml perfusate volume produced initial levels several-fold lower than expected (Table 2). Comparison of the administration of high-dose Cremophor in the presence and absence of a liver indicates that there is hepatic uptake of Cremophor. The perfusate levels decreased during the 3-h experiment regardless of the presence of a liver, possibly due to binding either to red cells or the tubing of the perfusate circuit.

Table 2 Perfusate concentrations of Cremophor in selected control experiments measured using a bioassay[a] (*conc.* Concentration)

Experiment		Cremophor perfusate conc. (mg/ml)			
		Sample time (min)			
Cremophor dose	Liver present	3	60	120	180
800 mg	No	6.0	3.0	1.6	1.0
800 mg	Yes	0.80	0.40	0.14	0.11
80 mg	Yes	0.30	0.04	0.02	0.01

[a] $n = 1$

Discussion

In the present study, Cremophor at clinically relevant concentrations inhibited etoposide elimination from the isolated perfused rat liver. Both the volume of distribution and the total clearance of etoposide were reduced in a dose-dependent manner, and biliary clearance was also significantly decreased, without affecting liver viability. Animal studies have shown that other MDR modulators such as verapamil and cyclosporine can change the pharmacokinetics and tissue distribution of doxorubicin [7, 27], and the cyclosporine analogue PSC 833 increases the blood levels and toxicity of etoposide [15]. There are numerous reports in the literature of clinical trials of MDR modulators given in combination with cytotoxic chemotherapy, and many of these investigations have conducted studies of the influence of the modulator on the pharmacokinetics of the cytotoxic drug. Cyclosporine increased the AUC and decreased the clearance of doxorubicin [2] and etoposide [19], and verapamil caused similar effects on doxorubicin elimination [16]. These pharmacokinetic changes may be due to inhibition by the MDR modulator of the P-glycoprotein transporter in normal tissues, resulting in decreased elimination of the cytotoxic drug.

It is possible that Cremophor is inhibiting P-glycoprotein (Pgp)-mediated biliary excretion of etoposide. Cremophor is known to modulate MDR in vitro, presumably by inhibiting the Pgp efflux pump [10, 23, 35], reaching maximal inhibition at a concentration of 1.0 mg/ml and losing all activity by 0.1 mg/ml. The effect is rapid and reversible and may be either due to a direct interaction with Pgp [10] or the result of a general membrane perturbation affecting its function [6, 24]. The Pgps are a family of energy-dependent

membrane transporters that can be separated into two functional groups. The protein responsible for the MDR phenotype is encoded by the *mdr1* gene in humans and by the *mdr1* and *mdr3* genes in rodents. Tissue-localization studies indicate that expression of this Pgp is high in specialized tissues such as haemopoietic stem cells, endothelial cells of the blood-brain barrier, and secretory epithelial cells in the kidney, adrenal, liver, and small intestine [30]. These specific locations of Pgp suggest that it has a role in cellular export of endogenous substances and xenobiotics. Many studies have provided indirect evidence that the Pgp located within the canalicular membrane is probably responsible for the biliary excretion of many compounds, including rhodamine 123, colchicine, doxorubicin, vinblastine and vincristine, since its function can be inhibited by classic Pgp inhibitors such as verapamil and cyclosporine [22, 25, 26, 28, 31]. It has recently been shown that *mdr2* expression in the mouse is largely restricted to the canalicular membrane of epithelial cells lining the bile canaliculi and biliary ductules [5]. Although the normal physiological function of this *mdr2* Pgp isoform is unknown, it does not confer resistance to drug-sensitive cells, but it has been shown to contribute to bile formation by mediating phospholipid transport [3, 22]. An effect of Cremophor on this Pgp is possible, although the consequences on the biliary excretion of etoposide are not clear.

In humans, approximately 30–40% of an intravenous dose of etoposide is excreted unchanged in urine, and less than 5% is recovered unchanged in bile [12]. The disposition of the remainder is unclear, but total recovery of radioactively labeled etoposide has recently been demonstrated in humans, with 56% being recovered in urine, mostly as the unchanged drug, and 44% being recovered in faeces [14]. This suggests that biliary excretion is a significant route of elimination. The glucuronide conjugate of etoposide has been identified as a potentially important metabolite [12], and previous studies in the isolated perfused rat liver have demonstrated that up to 98% of a dose of etoposide is excreted in the bile as approximately equal amounts of both etoposide and its glucuronide [11]. Although we did not measure the glucuronide, the biliary excretion of etoposide in our study was comparable with previous reports, and it is likely that the remainder of the dose was present in bile as the glucuronide.

It is unlikely that direct inhibition of cytochrome P450 metabolism by Cremophor contributed to the interaction, since this would cause the etoposide elimination half-life to increase, whereas in the present study, the terminal elimination half-life decreased. However, Cremophor may have altered the distribution of etoposide in the perfusate and, therefore, decreased or delayed liver uptake and, consequently, inhibited clearance. This would explain the large increase in perfusate AUC obtained with high-dose Cremophor and would resolve the apparent anomaly in the biliary excretion results. In particular, even though the overall cumulative biliary excretion of etoposide was not changed, the biliary clearance (dependent on the perfusate AUC and on total biliary excretion) was significantly lower following the addition of high-dose Cremophor. The biliary excretion results indicate that the effect of Cremophor is greatest during the 1st h, which coincides wtih the highest perfusate levels of Cremophor. The liver then recovers its ability to eliminate etoposide into the bile. In the presence of Cremophor, the etoposide volume of distribution decreased significantly and there was a change from biexponential to monoexponential etoposide elimination. One explanation for these distribution changes may be the incorporation of etoposide within Cremophor micelles. Kessel [17] has shown that daunorubicin is incorporated into Cremophor micelles, which form at Cremophor concentrations above 0.1 mg/ml. Micelles are thought to be capable of inhibiting hydrophobic drugs from precipitating, binding or undergoing enzymatic attack or renal excretion [9, 36]. The formation of Cremophor micelles encompassing etoposide might therefore explain the changes in etoposide distribution. This may also account for the recent findings of an in vivo partitioning study of colchicine in rat liver [25], showing that Cremophor inhibited colchicine hepatic elimination through reduced uptake across the basolateral membrane (from plasma to hepatocyte) rather than by decreased PgP excretion across the canalicular membrane (from hepatocyte to bile). In our study, the large increase in the etoposide perfusate AUC, resulting in decreased total and biliary clearance, together with the lack of a significant change in total biliary excretion, could be explained by impaired uptake into the liver either instead of or in addition to a transient effect of Cremophor on PgP-mediated biliary excretion.

Although only total etoposide was measured, it is possible that Cremophor affected plasma protein binding. Cremophor at concentrations of 1–3 mg/ml is known to associate in plasma preferentially with low-density lipoproteins, and at higher concentrations it destroys high-density lipoproteins [18]. This same group reported that Cremophor did not interact with human serum albumin. However, it has also been reported that Cremophor apparently alters the biodistribution of paclitaxel by decreasing the affinity to albumin and increasing its association with low-density lipoproteins [29]. Our perfusate contains bovine serum albumin in an artificial buffer, and the relevance of any potential effect of Cremophor on etoposide binding is not clear.

Other surfactants, including Tween 80, are capable of reversing MDR [10, 35], but many are also toxic to cells. Although Tween 80 is more potent than Cremophor (significant reversal of MDR occurs at 0.1 mg/ml, with peak activity occurring at 1.0 mg/ml), cellular integrity studies following a 1-h exposure indicate that this agent begins to cause toxicity at

0.1 mg/ml, whereas Cremophor causes similar toxicity at 10 mg/ml [35]. These results are consistent with the present finding that Tween 80 caused haemolysis and cholestasis, whereas the equivalent dose of Cremophor was non-toxic. Nevertheless, Tween 80 at a dose equivalent to the low dose of Cremophor had effects similar to those of low-dose Cremophor on etoposide pharmacokinetics. However, there are conflicting reports in the literature on the effects of Tween 80 on drug distribution and elimination. Tween 80 has been shown to increase the plasma levels of doxorubicin in mice, apparently by decreasing the plasma volume [13], whereas in patients it has increased the volume of distribution and clearance of doxorubicin and decreased the AUC [8]. Tween 80 is also known to facilitate the distribution of methotrexate into the brain and to increase its renal and biliary excretion [1], which contrasts with our finding of decreased biliary excretion of etoposide. These studies suggest that the surfactant properties of Tween 80, which may increase membrane permeability, are likely to prevent its usefulness as an MDR modulator.

We have previously shown that patients receiving paclitaxel as a 3-h infusion have peak plasma Cremophor levels of 1–2 mg/ml [32]. The present study indicates that these concentrations of Cremophor may influence the pharmacokinetics of etoposide. A clinical trial of the combination of paclitaxel and etoposide, including an evaluation of the influence of paclitaxel on the pharmacokinetics of etoposide, is now in progress. The implications of coadministration of the current formulation of paclitaxel with other cytotoxics should also be considered, and the results of this study suggest that the clinical use of Cremophor to circumvent MDR should involve close monitoring of the pharmacokinetics and toxicities of the cytotoxic drug [20]. Nevertheless, as compared with other MDR modulators, the relative lack of toxicity of Cremophor indicates that it may be a potentially useful chemosensitizer.

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