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Modulation of cisplatin pharmacodynamics by Cremophor EL: experimental and clinical studies

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Abstract

The paclitaxel vehicle Cremophor EL (CrEL) has been shown to selectively inhibit the accumulation of cisplatin in peripheral blood leucocytes, but not in tumour cells *in vitro*, and we hypothesised that this phenomenon is responsible for the improvement of the therapeutic index of cisplatin observed in combination studies with paclitaxel. Here, we report on studies assessing the interaction between CrEL and cisplatin in a murine model, and involving the potential clinical applicability of CrEL as a protector for cisplatin-associated haematological side-effects. In mice, CrEL (0.17 ml/kg, intravenous (i.v.)) given in combination with cisplatin (10 mg/kg, intraperitoneal (i.p.)) did not change the pharmacokinetics of cisplatin. Cisplatin-induced haematological toxicity, expressed as white blood cells (WBC) at nadir, was significantly reduced by CrEL from 5.05 ± 0.95 to $6.50 \pm 1.31 \times 10^9$/l ($P = 0.0009$). Data obtained from cancer patients treated with cisplatin (70 mg/m^2, 3-h i.v.) and topotecan (0.45 or 0.60 mg/m^2/day$\times$2) preceded by CrEL (12 ml, 3-h i.v.) ($n = 6$) or without CrEL ($n = 10$) similarly indicated significant differences in the percent decrease in WBC between the groups (46.5 ± 18.7 versus $67.2 \pm 15.0\%$; $P = 0.029$). Likewise, the percent decrease in platelet count was significantly greater in the absence of CrEL (23.9 ± 5.38 versus $73.3 \pm 15.5\%$; $P = 0.0003$). Pharmacokinetic parameters of unbound and total cisplatin and of topotecan lactone and total drug were not significantly different from historic control values ($P \geqslant 0.245$). Overall, this study provides further evidence on the important role of CrEL in the pharmacological and toxicological profile of cisplatin, and implies that reformulation of cisplatin with CrEL for systemic treatment might achieve an improvement of its therapeutic index, particularly in the setting of a weekly dose-dense concept.

Keywords: Cisplatin; Pharmacokinetics/dynamics; Myelosuppression; Cremophor EL

1. Introduction

Cisplatin is one of the most frequently used drugs in the treatment of a wide variety of solid tumours, including testicular cancer, ovarian cancer, (non-)small cell lung cancer, head and neck cancer and bladder cancer [1]. Major side-effects of the conventional 3-weekly application are nausea/vomiting, renal toxicity, neurotoxicity and ototoxicity. There is a substantial body of evidence that for cisplatin, similar to other cytotoxic agents, the dose–response relationship is steep. Clinical studies have indicated in tumour types such as head and neck cancer and ovarian cancer that higher dose intensity, if achievable, leads to a higher response rate [2]. Most frequently, reaching a higher dose-intensity was attempted by increasing the dose per administration. However, in practice this led to unacceptable side-effects, preventing the further use of this strategy [3]. Another way of increasing dose intensity is by shortening the treatment intervals without changing the dose per administration. Since 1988, we have been performing a large clinical and preclinical programme focusing on interval shortening for cisplatin, with the drug given weekly. In a phase I study, the maximum tolerable dose was found to be 80 mg/m^2/week. Dose-limiting toxicity was myelosuppression, while the commonly known side-effects of cisplatin were not dose-limiting using this schedule [4]. Phase II studies have

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shown major activity in head and neck cancer [5], and even in a tumour type such as mesothelioma that is commonly considered to be chemotherapy resistant [6]. By adding oral etoposide to the weekly administration of cisplatin [7], high response rates were also obtained in non-small cell lung cancer [8], and even in ovarian cancer resistant to conventional cisplatin schedules [9]. Recently, we have added weekly administered Taxol (i.e. paclitaxel formulated in Cremophor EL (CrEL)–ethanol, 1:1, v/v) to our regimen of weekly cisplatin, and observed a marked reduction in myelosuppression compared with what would have been expected based upon the single agent toxicities ([10] and data not shown). Similar observations have been made in clinical studies with conventional 3-weekly schedules [11]. In general, the pharmacokinetics of both drugs during the two tested sequences (paclitaxel preceding and following cisplatin) was unaltered, indicating that a pharmacodynamic interaction must have occurred. Up until now, convincing data regarding the fundamental reasons for these clinically important pharmacodynamic interactions are lacking. Based upon previous *in vitro* work indicating selective inhibition of cisplatin accumulation by CrEL in peripheral white blood cells (WBCs), but not in tumour cells [12,13], we speculated that CrEL is responsible for the improvement of the therapeutic index of cisplatin observed in combination studies with paclitaxel. Here, we report on studies assessing the interaction between CrEL and cisplatin in a murine model, and involving the potential clinical applicability of CrEL as a protector for cisplatin-associated haematological side-effects.

2. Materials and methods

2.1. Chemicals

Cisplatin (Platosin) was purchased as a powder from Pharmachemie (Haarlem, The Netherlands). Solutions of cisplatin for experimental use were prepared in isotonic saline at a concentration of 10 mg/ml. CrEL (lot 16H0043) was obtained from Sigma Chemical Co. (St. Louis, MO, USA), and topotecan reference material was kindly provided by SmithKline Beecham (Harlow, UK). All other chemicals and reagents were of analytical grade or high performance liquid chromatography (HPLC) grade and were obtained from Rathburn (Walkerburn, UK). Water was filtered and deionised by the Milli-Q-UF Plus system (Millipore, Bedford, MA, USA) and was used in all aqueous solutions.

2.2. In vitro *blood distribution*

Aliquots (1 ml) of freshly prepared heparinised human whole blood were incubated for 15 min at 37 °C with 5 ng/ml of topotecan lactone (L) and 5 ng/ml of topotecan carboxylate (C) in the combination with CrEL at final whole blood concentrations of 0, 0.5, 1.0, 2.0 and 4.0 μl/ml. Subsequently, the samples were centrifuged for 5 min at 2500*g* (4 °C) to separate the plasma. The plasma samples were directly deproteinised with a 3-fold volume of ice-cold (−20 °C) methanol, and were stored at −80 °C prior to analysis. The lactone to total drug concentration ratio in the plasma was calculated as: L/(L + C). The remaining plasma was used for the determination of the CrEL concentrations.

In a separate set of experiments, a volume of 5 ml whole blood was incubated with 5 μg/ml of cisplatin in the absence and presence of CrEL. Aliquots of 1 ml were centrifuged for 5 min at 2500*g* (4 °C) for the measurement of total and unbound platinum concentrations in the plasma compartment at the following timepoints: immediately after start of the incubation, and at 0.5, 1 and 2 h after the start of incubation. For the determination of the unbound cisplatin concentrations, 500 μl aliquots of the plasma supernatant were added to 1000 μl of ice-cold (−20 °C) ethanol directly after collection of the plasma, and were stored at −80 °C prior to analysis.

2.3. Animal studies

Female FVB non-tumour bearing mice (10–12 weeks of age; weight, 23.2 ± 1.87 g) were obtained from Harlan Nederland (Horst, The Netherlands), and were used in all experiments. The animals were divided into groups of eight mice, and placed in methacrylate cages covered with filter bonnets in a controlled environment maintained on an automatic 12-h lighting cycle at a temperature of 22 ± 2 °C according to institutional guidelines. The animals were given a standard chow (Hope Farms B.V., Woerden, The Netherlands) and acidified water *ad libitum*. All solutions were prepared within 1 h prior to injection and stored on ice until use. Mice in groups of 5–20 were injected intravenously (i.v.) under light diethyl ether anaesthesia with isotonic saline or a CrEL solution containing 50 μl/ml in isotonic saline (injection volume, 100 μl/30 g body weight). Administration was performed by injection into the tail vein after the mice had been gently warmed under a red 100-W lamp and had their tails soaked briefly in a warm water bath. The mice were then randomly divided into two groups; at 1 h after the first administration, one group was injected intraperitoneally (i.p.) with a cisplatin solution containing 1 mg/ml in isotonic saline (injection volume, 300 μl/30 g body weight), and the other group was injected with isotonic saline as a control. The final CrEL and cisplatin doses were 0.17 ml/kg and 10 mg/kg, respectively. The cisplatin dose was previously determined as the i.p. dose that results in death of 50% of mice within 9 days [14], and was chosen

based on preliminary experiments (using four mice per dose level each at 0, 2.5, 5.0 and 10 mg/kg) indicating that it would result in a substantial degree of myelosuppression (%decrease in WBC at nadir: 25.9, 31.8 and 55.5, respectively at 2.5, 5.0 and 10.0 mg/kg). Mice were bled under light diethyl ether anaesthesia at approximately 10 a.m. on the days specified. Blood samples of 40 μl were obtained from the tail vein (days 1–4) or the retro-orbital venous plexus (day 5) in a heparinised glass microtube before the daily determination of peripheral blood-cell counts using an autoanalyser. For pharmacokinetic purposes, additional blood samples were obtained from mice at 5, 15, 30 and 45 min, and 1, 2, 4, 6, 8, 16, and 24 h after the i.v. CrEL administration (dose, 0.17 ml/kg body weight), using four animals per time point, into polypropylene microtubes containing 7 USP units of lithium heparin as an anticoagulant. Similarly, blood samples were taken on the third, fourth and fifth day following i.p. cisplatin administration both in the absence and presence of CrEL. These samples were placed on ice, and plasma was separated within 10 min by centrifugation for 5 min at 2500*g* (4 °C), and then stored at −20 °C until analysis. It was confirmed that the observed concentrations of cisplatin and CrEL in all dosing solutions were within ±5% of their nominal target values. The experimental protocol was approved by the ethical committee of the Animal Welfare Office (Erasmus University, Rotterdam, The Netherlands).

2.4. Clinical studies

Patients with recurrent or progressive ovarian cancer were treated with six cycles of weekly cisplatin (70 mg/m^2 infused over 3 h) with or without CrEL (12 ml), in combination with oral topotecan (0.45 or 0.60 mg/m^2/day for 2 days). CrEL was administered as a 3-h i.v. infusion immediately prior to cisplatin, and the dose was similar to that administered with paclitaxel at a dose level of 90 mg/m^2. Blood samples for pharmacokinetic analysis were obtained from an indwelling i.v. canula and collected in vials containing lithium heparin as anticoagulant at the following timepoints: prior to dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after topotecan administration; and at 1, 2, 3, 3.5, 4, 5, 6 and 21 h after the start of the cisplatin infusion. Blood samples for the assessment of haematological parameters were obtained (at least) on a weekly basis. The protocol was approved by the institution's Medical Ethical Committee and all patients signed an informed consent form.

2.5. Pharmacological analysis

The analytical assay for unbound cisplatin and total cisplatin was based on flameless atomic absorption spectrometry [15]. Simultaneous determination of the lactone and carboxylate forms of topotecan was performed by reversed-phase HPLC with fluorescence detection [16], with minor modifications as described in Ref. [15]. CrEL concentrations in plasma samples were measured using a colorimetric dye-binding microassay [17]. The plasma concentration–time profiles of unbound and total cisplatin were fitted to two-compartmental linear models with extended least-squares regression analysis using the Siphar v4.0 software package (Innaphase, Champs-sur-Marne, France). The area under the plasma concentration–time curve (AUC) of cisplatin was calculated to the last sampling timepoint with detectable drug levels (C_{last}) by the linear trapezoidal method, and extrapolated to infinity by the addition of C_{last}/k_{term}, where k_{term} is the slope obtained by log-linear regression analysis of the final plasma concentration values. Unbound cisplatin clearance was calculated by dividing the administered dose by the observed AUC, and the terminal disposition half-life was calculated as $\ln 2/k$, where k is the rate constant of the terminal disposition phase. The peak plasma concentration was determined graphically from the observed experimental values. The potential for drug accumulation or altered disposition following repeated cisplatin administration

Table 1
Effect of CrEL on *in vitro* cisplatin concentrations in plasma[a]

CrEL (μ/ml)	AUC_u (μg h/ml)	$T_{1/2}$ (h)	AUC_u/AUC_{tot}	$fu_{2\text{-h}}$
0	6.39±0.263	1.37±0.06	0.581±0.013	0.349±0.008
0.5	6.35±0.161	1.36±0.04	0.573±0.026	0.338±0.010
1.0	6.33±0.220	1.35±0.01	0.573±0.006	0.338±0.009
2.0	6.49±0.146	1.35±0.042	0.582±0.016	0.347±0.020
4.0	6.32±0.230	1.30±0.025	0.577±0.005	0.345±0.010
	$P=0.83$[b]	$P=0.31$[b]	$P=0.91$[b]	$P=0.99$[b]

AUC, area under the plasma concentration–time curve; $T_{1/2}$, disappearance half-life; u, unbound cisplatin; tot, total cisplatin; $fu_{2\text{-h}}$, fraction unbound cisplatin in plasma at 2 h after start of incubation; CrEL, Cremophor EL.

[a] Data were obtained at several timepoints after incubating cisplatin (5 μg/ml) in whole blood in the absence and presence of CrEL, followed by centrifugation and analysis of the plasma supernatant. Data are presented as mean values of at least three independent observations per group ±standard deviation (S.D.).

[b] One-way analysis of variance test.

was assessed by the ratio of AUC values between treatment courses (i.e. AUC_{c1}/AUC_{c2} and AUC_{c1}/AUC_{c3}). For topotecan, the concentration-time profiles were obtained after zero-order input with weighted least-squares regression analysis applying a weight factor of $1/y$. The AUCs of the lactone (AUC_L) and carboxylate (AUC_C) forms were determined on the basis of the best fitted curves, and the apparent clearance of topotecan lactone (CL/f) was determined using the dose expressed in free base equivalents and the fitted AUC. Other parameters were assessed as outlined for cisplatin. The ratio of the systemic exposure of topotecan lactone to total drug (L/T ratio) was defined as $AUC_L/(AUC_L + AUC_C)$.

3. Results

3.1. Preclinical evaluation

Prior to the *in vivo* testing, it was confirmed that CrEL at clinically relevant concentrations had no effect on the blood distribution and plasma protein binding of cisplatin in human samples *in vitro* ($P > 0.3$ at the five tested concentrations; Table 1). In addition, CrEL did not substantially influence erythrocyte partitioning and the lactone to carboxylate interconversion of topotecan at similar concentrations (Table 2), although the topotecan lactone concentration was slightly reduced at a CrEL concentration of 1.0 μl/ml by one-way ANOVA. This effect was not consistently observed with increased CrEL levels, and was considered of minor importance, particularly in view of substantial kinetic variability with the planned topotecan regimen [18].

Table 2
Effect of CrEL on *in vitro* topotecan concentrations in plasma[a]

CrEL (μl/ml)	$C_{15\text{-min}}$ L (ng/ml)	$C_{15\text{-min}}$ C (ng/ml)	L/T ratio
0	3.33±0.108	9.65±0.138	0.257±0.0037
0.5	3.19±0.057	9.52±0.099	0.251±0.0018
1.0	3.10±0.018	9.43±0.172	0.247±0.0045
2.0	3.13±0.065	9.37±0.114	0.251±0.0056
4.0	3.20±0.085	9.45±0.370	0.253±0.0026
	$P = 0.023$[b,c]	$P = 0.51$[b]	$P = 0.14$[b]

$C_{15\text{-min}}$, topotecan concentration in plasma at 15 min after start of incubation; L, topotecan lactone form; C, topotecan carboxylate form; T, topotecan total drug (lactone plus carboxylate forms); CrEL, Cremophor EL.

[a] Data were obtained after incubating topotecan lactone and carboxylate forms (5 ng/ml each) in whole blood for 15 min in the absence and presence of CrEL, followed by centrifugation and analysis of the plasma supernatant. Data are presented as mean values of at least three independent observations per group±standard deviation (S.D.).

[b] One-way analysis of variance test.

[c] Control (CrEL concentration, 0 μl/ml) significantly different from the other groups.

The CrEL dose tested in our murine model was shown to be associated with plasma concentrations in the range of 0.5–3.0 μl/ml (Fig. 1a), which is within the range required to modulate cellular cisplatin accumulation in peripheral blood leucocytes *in vitro* [12,13]. Cisplatin given alone (10 mg/kg, i.p.) induced a significant reduction in the peripheral WBC count at nadir (39.4%), compared with saline-treated control mice ($P < 0.0001$), similar to previous findings [20]. The addition of CrEL resulted in significant protection against cisplatin-induced haematological toxicity as observed from increased WBC nadir values (Table 3). CrEL given alone had no effect on any of the studied parameters (Table 3).

3.2. Clinical studies

Complete pharmacokinetic data were obtained from 3 patients treated with cisplatin and oral topotecan at 0.45 mg/m^2/day×2, from 3 patients treated with the same regimen preceded by a 3-h i.v. infusion of CrEL

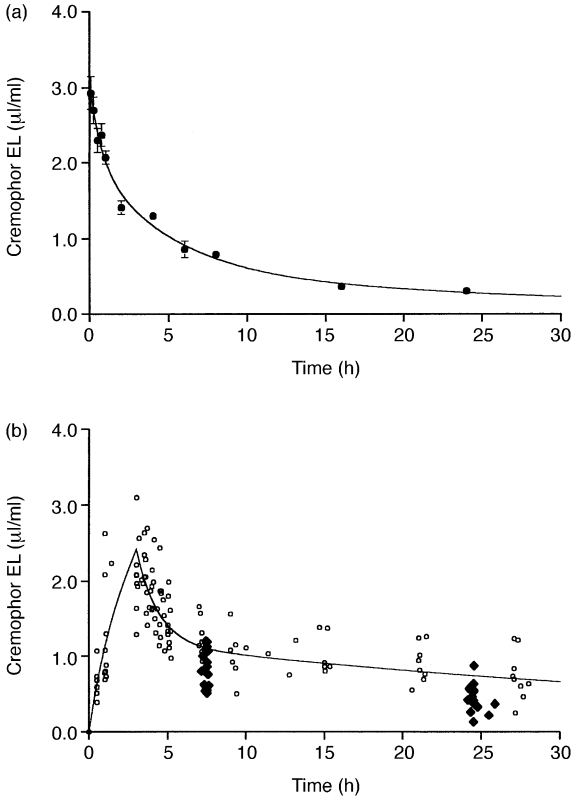

Fig. 1. (a) Plasma concentration-time profile of Cremophor EL (CrEL) in female FVB mice after intravenous (i.v.) administration of CrEL at 0.17 ml/kg; data are displayed as mean values (symbols)± standard deviation (S.D.) (error bars); (b) plasma concentration–time profiles of CrEL in 6 patients (closed symbols) treated with 3-h i.v. infusion of CrEL (dose, 12 ml) followed by cisplatin (dose, 70 mg/m^2) and topotecan (dose, 0.45 or 0.60 mg/m^2/day×2), and in 11 patients (open symbols and curve fit) receiving single agent paclitaxel formulated in CrEL–ethanol–isotonic saline at a dose of 100 mg/m^2 (CrEL dose, 8.33 ml/m^2) (unpublished data) [20].

(12 ml), and from 3 patients treated with the same combination after topotecan dose escalation (0.60 mg/m^2/day×2). Cisplatin pharmacokinetic parameters were compared with data from 76 patients treated at the Rotterdam Cancer Institute with single agent cisplatin (70 mg/m^2) [21]. No statistically significant differences were found in the parameters for both unbound and total cisplatin between the control and CrEL-treated groups (Table 4 and Fig. 2a). In addition, topotecan lactone and total drug pharmacokinetic profiles were also unaffected by CrEL when compared with single agent topotecan (Table 4 and Fig. 2b). The observed plasma concentrations of CrEL following its administration were in the same range as previously described (Fig. 1b) [20].

Comparative haematological pharmacodynamic data obtained from treatment courses with ($n=6$) or without CrEL ($n=10$) indicated that the percent decrease in WBC was significantly reduced in the CrEL-treated group at the same topotecan dose (46.5±18.7 versus 67.2±15.0%; $P=0.029$) (Table 5). Likewise, the percent decrease in platelet count was significantly worse in the absence of CrEL (23.9±5.38 versus 73.3±15.5%; $P=0.0003$).

Table 3
Haematological toxicity of cisplatin in a mouse model[a]

Treatment group	WBC nadir (×10^9/l)	ANC nadir (×10^9/l)	PLC nadir (×10^9/l)	HT (l/l)
Saline	8.33±1.82	0.90±0.76	398±419	0.435±0.007
	(7.12–11.5)	(0.20–1.51)	(29–1057)	(0.427–0.443)
CrEL	9.52±2.40	0.74±0.36	296±205	0.427±0.031
	(6.99–13.4)	(0.14–1.39)	(17–550)	(0.382–0.485)
Cisplatin	5.05±0.95[b,c]	0.64±0.42	484±293	0.411±0.063
	(3.22–6.93)	(0.14–2.04)	(28–1200)	(0.223–0.504)
Cisplatin + CrEL	6.50±1.31[d]	1.10±0.89	383±240	0.436±0.031
	(5.10–9.35)	(0.31–3.33)	(72–776)	(0.392–0.492)

WBC, white blood cell count; ANC, absolute neutrophil count; PLC, platelet count; HT, haematocrit; CrEL, Cremophor EL.

[a] Data were obtained on the third, fourth or fifth day after administration of cisplatin (10 mg/kg, i.p.) with or without CrEL (0.17 ml/kg, i.v.) to female FVB mice. Data are presented as mean values of 5–20 observations per group±standard deviation (S.D.), with the observed range shown in parentheses.

[b] Significantly different from the saline group at $P<0.0001$.

[c] Significantly different from the CrEL group at $P<0.0001$.

[d] Significantly different from the cisplatin group at $P=0.0009$.

Table 4
Summary of cisplatin and topotecan pharmacokinetics in the presence and absence of CrEL[a]

Parameter	With CrEL	Without CrEL[b]	P value[c]
Cisplatin			
C_{max} (μg/ml)	0.90±0.22	0.81±0.18	0.249
AUC_{fu} (μg h/ml)	3.00±1.52	2.34±0.43	0.338
CL_{fu} (l/h/m^2)	32.2±24.8	31.1±6.63	0.771
fu/tot ratio	0.082±0.045	0.076±0.013	0.403
AUC_{c1}/AUC_{c2}	0.886±0.528	0.774±0.049	0.626
AUC_{c1}/AUC_{c3}	0.930±0.590	0.718±0.128	0.420
Topotecan			
CL/f_L (l/h/m^2)	142±95.7	107±22.5	0.448
L/T ratio	0.36±0.05	0.36±0.02	0.999
AUC_{c1}/AUC_{c2}	0.883±0.503	0.716±0.192	0.504
AUC_{c1}/AUC_{c3}	0.692±0.198	NA	NA

C_{max}, peak plasma concentration; AUC, area under the plasma concentration–time curve; c1-3, course 1-3; fu, fraction unbound cisplatin; CL, total plasma clearance; tot, total cisplatin (unbound plus bound fractions); c, treatment course number; CL/f. apparent oral clearance; L, topotecan lactone form; T, topotecan total drug (lactone plus carboxylate forms); NA, not available; CrEL, Cremophor EL.

[a] Data were obtained from 6 patients treated with cisplatin (dose, 70 mg/m^2) and topotecan (dose, 0.45 or 0.60 mg/m^2/day×2) preceded by a 3-h i.v. infusion of CrEL (dose, 12 ml) ($n=6$) Data are from day 1 of the first course and presented as mean values±standard deviation (S.D.).

[b] Data from 76 patients treated at the Rotterdam Cancer Institute with single agent cisplatin at a dose level of 70 mg/m^2 [21] and data from Creemers and colleagues [41] and Gerrits and colleagues [42] from 5 patients treated with single agent oral topotecan at a dose level of 0.50 mg/m^2.

[c] Unpaired two-tailed Student's t-test.

Table 5
Summary of haematological pharmacodynamics[a]

Parameter	With CrEL		Without CrEL
Topotecan dose	0.45 mg/m^2	0.60 mg/m^2	0.45 mg/m^2
Leukocytes			
Nadir ($\times 10^9$/l)	3.17±1.42 (2.3–4.8)	2.57±0.78 (1.7–3.2)	2.13±0.98 (0.41–3.4)
%decrease WBC	46.5±18.7 (27.3–72.7)	70.5±6.77 (65.4–78.2)	67.2±15.0[b] (46.8–92.5)
Neutrophils			
Nadir ($\times 10^9$/l)	1.10±0.20 (0.9–1.3)	0.70±0.46 (0.3–1.2)	0.83±0.63 (0.1–1.9)
%decrease ANC	69.0±11.0 (59.3–80.9)	88.0±7.59 (80.0–95.1)	81.8±13.1 (63.6–97.4)
Thrombocytes			
Nadir ($\times 10^9$/l)	73.3±7.51 (69–82)	57.0±24.2 (43–85)	79.7±59.5 (13–188)
%decrease PLC	23.9±5.38 (19.5–29.9)	75.4±10.3 (63.5–81.5)	73.3±15.5[c] (45.5–93.8)

WBC, white blood cell count; ANC, absolute neutrophil count; PLC, platelet count; CrEL, Cremophor EL.

[a] Data were obtained from 6 patients treated with cisplatin (dose, 70 mg/m^2) and topotecan (dose, 0.45 or 0.60 mg/m^2/day×2) preceded by a 3-h intravenous (i.v.) infusion of Cremophor EL (CrEL) (dose, 12 ml) ($n=6$) or without CrEL ($n=10$) [43]. The relative haematological toxicity (i.e. the percentage decrease in blood cell count) was defined as: %decrease=[(pretherapy value−nadir value)/(pretherapy value)]×100%. Data are presented as mean values±standard deviation (S.D.), with the observed range shown in parentheses.

[b] Significantly different from the 0.45 mg/m^2/day×2 plus CrEL group at $P=0.029$.

[c] Significantly different from the 0.45 mg/m^2/day×2 plus CrEL group at $P=0.0003$.

4. Discussion

The present study provides further evidence of the important role of the paclitaxel vehicle CrEL in the pharmacology and toxicology of cisplatin, and suggests that CrEL is responsible for the pharmacological interactions observed previously between cisplatin and paclitaxel in cancer patients [10]. Apart from the reduction of cisplatin-induced nephrotoxicity by saline infusions and forced hydration, the selective modulation of side-effects associated with cisplatin chemotherapy, notably myelotoxicity in dose-dense regimens, has not yet resulted in a substantially improved therapeutic index of the drug [1]. Studies in mice showed that CrEL given in addition to cisplatin did not decrease the antitumour activity [19]. Our hypothesis that CrEL may act as a modulator of cisplatin-associated myelotoxicity emerged directly from its use as haematopoietic-protecting agent in irradiated mice and our own preclinical and clinical experience.

Indeed, it has repeatedly been confirmed that the pharmaceutical vehicles present in paclitaxel and docetaxel formulations (Cremophor EL and Tween 80, respectively) may have a major impact on the pharmacology of concomitantly administered drugs. The influence of both vehicles on the reduced cisplatin uptake in cells in a previous study was readily shown from experiments with WBC incubated with CrEL or Tween 80 prior to cisplatin [12]. A significant reduction in the intracellular cisplatin concentration in WBC of up to 42% compared with the control was observed in the presence of CrEL or Tween 80. Furthermore, cisplatin accumulation was unaltered with paclitaxel or docetaxel formulations which did not contain CrEL or Tween 80 (viz. dimethylsulphoxide), suggesting that the inhibition of cisplatin accumulation in WBC is exclusively caused by the formulation vehicles. We now show that the CrEL concentrations used in these experiments, viz. 0.1 and 0.01 μl/ml, are readily achievable in murine and patient plasma for extended time periods after its administration, in accordance with previous data [20,22]. Thus, although not investigated in the current study, altered adduct formation with critical structures of bone marrow cells, might have contributed to the observed decrease in cisplatin-induced haematological toxicity in the presence of CrEL.

As an alternative mechanism underlying the decrease in the haematological toxicity, a number of reports published on the effects of CrEL administration on haematopoiesis *in vivo* may be particularly relevant. The first significant observation was the increase in circulating platelet levels recorded following prolonged administration of high doses of CrEL in dogs [23]. In contrast, in mice it was shown that peripheral blood cell parameters, including WBCs, reticulocyte and platelet counts are unaffected by CrEL administration, which is consistent with our current findings. However, i.v. administration of CrEL in mice was associated with a decrease in femoral bone marrow cellularity, an upregulation of B220 (B cells) and 7/4 surface antigen

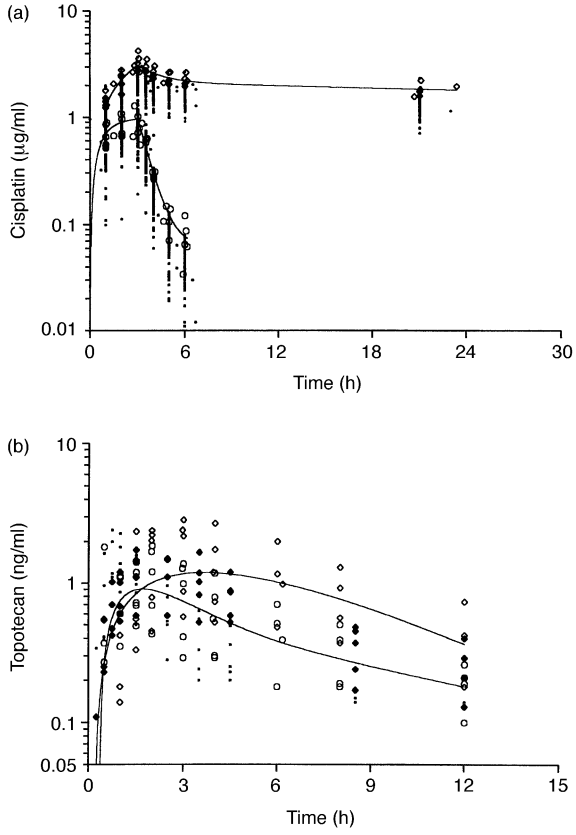

Fig. 2. (a) Plasma concentration–time profiles of unbound cisplatin (circles) and total cisplatin (triangles) in 6 patients (open symbols and curve fits) treated with a 3-h intravenous (i.v.) infusion of Cremophor EL (CrEL) (dose, 12 ml) followed by cisplatin (dose, 70 mg/m^2) and topotecan (dose, 0.45 or 0.60 mg/m^2/day×2), and in 76 patients (closed symbols) receiving single agent cisplatin at a dose of 70 mg/m^2 [21]; (b) plasma concentration–time profiles of topotecan lactone (circles) and carboxylate forms (triangles) in 6 patients (open symbols and curve fits) treated with a 3-h i.v. infusion of CrEL (dose, 12 ml) followed by cisplatin (dose, 70 mg/m^2) and topotecan (dose, 0.45 or 0.60 mg/m^2/day×2), and in 5 patients (closed symbols) receiving single agent topotecan at a dose of 0.50 mg/m^2.

expression (neutrophil and activated macrophage) in the marrow and an increase in the incidence of both primitive and committed progenitors [24]. Furthermore, CrEL protected mice from irradiation-induced death if administered prior to the dose [24]. This intriguing effect is consistent with the interpretation that CrEL activates accessory cells, and modulates accessory factors regulating haematopoietic progenitor cells through the operation of cytokine cascades. The induction of histamine release may also play a role in the haematopoietic response to CrEL administration [25–27] . Histamine is known to trigger colony forming units into the cell cycle stimulate the proliferation of committed haematopoietic progenitor cells [28,29], and modulate the response of primitive haematopoietic cells to interleukin-3 [30,31]. In mice, no evidence was obtained of localised toxicity or marrow destruction as a result of CrEL injection [24], which is consistent with flow cytometric studies demonstrating that even very high concentrations of CrEL (>10%) did not lyse mammalian cell membranes [32]. In addition, no other obvious effects attributable to CrEL have been noted. In this context, it is also particularly noteworthy that CrEL has been shown to reduce haematological toxicity profiles in mice following the administration of several other chemotherapeutic agents, including carboplatin and 5-fluorouracil [33]. Clearly, additional studies on the combined use of CrEL with cisplatin at the molecular, cellular and *in vivo* level are desired for a better understanding of this clinically potentially important phenomenon.

One important observation from this work is that CrEL does not significantly alter the pharmacokinetic behaviour of cisplatin and topotecan, thus ruling out kinetic modifications that might explain the altered toxicity of the combination. Pharmacokinetic studies in patients have revealed that CrEL selectively distributes within the central blood/bone marrow compartment and exists in plasma as large polar micelles [22,34]. In addition, we have demonstrated previously that CrEL can have a profound effect on the pharmacokinetic behaviour of paclitaxel, resulting in a non-linear increase in the AUC [35]. Apparently, in the presence of CrEL, paclitaxel prefers to stay within the plasma compartment, which may be due to inclusion and partitioning in micelles [34]. Recent experiments with doxorubicin [36], etoposide [37], and the photosensitiser C8KC [38], indicate that this effect may not be unique for paclitaxel. If micellar encapsulation of these agents by CrEL is the sole explanation for the kinetic interactions, then it can be anticipated that water-soluble agents such as cisplatin and topotecan are not readily incorporated into the highly hydrophobic interior of CrEL micelles [39], and subsequently do not change the pharmacokinetic profiles.

One limitation of our study is the additional administration of topotecan, which itself is a highly myelotoxic agent, to the tested regimen in the patients [15,40]. Therefore, we cannot rule out the possibility that CrEL only protected from cisplatin-induced haematological toxicity and that topotecan-mediated side-effects might have influenced the overall clinical benefit. However, in spite of this drawback, it can be speculated in the light of the current observations that modulation of cisplatin pharmacodynamics alone is sufficient to improve pharmacodynamic outcome of the combination. Future studies are planned to confirm the current findings using a randomised clinical study in combination with dose escalation of CrEL to define the optimal dose. Based on previous clinical experience, it is expected that doses up to 30 ml/m^2 as a 3-h i.v. infusion can be safely administered without the occurrence of hypersensitivity reactions [36]. This type of study may lead to the clinical use of CrEL as an inexpensive protective agent of cisplatin-associated haematological toxicity.

In conclusion, this study provides further evidence of the important role of CrEL in the modulation of the pharmacological and toxicicological profile of cisplatin, and implies that reformulation of cisplatin with CrEL for systemic treatment might achieve improvement of its therapeutic index, particularly in the setting of a weekly dose-dense concept.

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