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Real-time pharmacokinetics guiding clinical decisions: phase I study of a weekly schedule of liposome encapsulated paclitaxel in patients with solid tumours☆

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Abstract

The purpose of this weekly schedule phase I study of liposome encapsulated paclitaxel (LEP) was to define the maximum-tolerated dose (MTD), the recommended dose (RD), the dose-limiting toxicities (DLTs), the pharmacokinetic profiles, and to evaluate preliminarily antitumour effects in patients with refractory solid malignancies. LEP was administered as an intravenous (i.v.) infusion over 45 min once every week for 6 out of 8 weeks. Fourteen patients were treated at doses ranging from 90 to 150 mg/m^2/week. In one patient, DLT was observed at the dose level of 150 mg/m^2/week, who received less than 70% of the intended cumulative dose. No cumulative toxicities were observed. Stabilisation of disease for 8 weeks was documented in two patients. The whole blood clearance of total paclitaxel was similar for LEP (15.3±8.98 l/h/m^2) and Taxol® (17.5±3.43 l/h/m^2), and the extraliposomal to total drug ratio increased rapidly to unity at later sampling time points.. The trial was discontinued upon completion of enrolment of the 150 mg/m^2/week cohort because an assessment of the pharmacokinetics and clinical data suggested that LEP was unlikely to have any advantages over Taxol®. It is concluded that this formulation of LEP is unlikely to provide improvements over the taxanes currently in clinical use.

Keywords: Paclitaxel; Liposome; Drug delivery system; Phase I; Pharmacokinetics

1. Introduction

Paclitaxel, a complex diterpenoid natural product derived from the bark of the Western yew tree, *Taxus brevifolia*, belongs to the class of anti-microtubule agents and is active in a broad variety of human malignancies, including breast, ovarian and non-small cell lung cancer. Due to the agent's poor solubility in aqueous solutions, paclitaxel is formulated for clinical use in a mixture of Cremophor EL and ethanol (Taxol®). Previous work has indicated that Cremophor EL contributes to the non-linear pharmacokinetic behaviour of paclitaxel and to severe hypersensitivity reactions in humans observed after the administration of Taxol® [1,2]. The incidence of these severe hypersensitivity reactions is approximately 41% despite the use of premedication with corticosteroids and anti-histamines (see: http://www.taxol.com). It has been proposed that the hypersensitivity reaction to Taxol® is caused by a Cremophor EL-mediated activation of the complement system [3]. The clinical formulation of Taxol® has also been associated with other side-effects, including peripheral neurotoxicity [4].

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To overcome the problems associated with the current formulation of paclitaxel, several chemical, pharmaceutical, and/or biological strategies are being explored to optimise chemotherapeutic treatment with paclitaxel [5]. One of the strategies is delivery of the drug by the use of liposomes (ranging in size from 10 nm to 20 μm) consisting of an aqueous core surrounded by one or more membranes consisting of naturally or synthetic phospholipids arranged in a bilayer configuration [6,7]. These spherical vesicles can encapsulate various therapeutic agents, including anticancer agents [8]. One rationale for encapsulating cytotoxic drugs in liposomes is based on the hypothesis that macromolecular (liposomal) carrier leakage will occur in tumour tissue due to its enhanced permeability and retention (EPR) effect [9,10]. This EPR effect is caused by discontinuation of the endothelium of tumour blood vessels, as a result of structural and functional anomalies, and the co-existing lack of a fully functional system of lymphatic drainage [11]. The interplay between these characteristics of tumour tissue can result in the extravasation and retention of liposomes within the tumour interstitium, with the potential for providing more active drug to the tumour with less exposure to normal tissue.

Since liposome encapsulation is suitable for the intravenous (i.v.) delivery of poorly water-soluble compounds, paclitaxel has also been proposed for administration in liposomes [8]. A liposome formulation (without Cremophor EL) could have considerable potential given the problems associated with Cremophor EL. Several liposome-based formulations of paclitaxel have been tested *in vivo* for antitumour activity in various models [12–15], and for various liposome formulations the maximum tolerated dose (MTD) was 2- to 7-fold greater than for Taxol® [12].

The encapsulation of cytotoxic agents into liposomes (e.g. anthracyclines) has been shown to substantially modulate the pharmacokinetic behaviour of these drugs [16]. This approach may enhance the efficacy of anticancer drugs and reduce their systemic toxicity through the lower exposure of normal tissues to the drug. The aim of this study was to define the MTD, recommended dose (RD), dose-limiting toxicities (DLTs), pharmacokinetic profiles, and evaluate preliminarily antitumour effects of a weekly schedule of liposome encapsulated paclitaxel (LEP) in patients with refractory solid malignancies.

2. Patients and methods

2.1. Patient selection

Patients with a histologically-confirmed diagnosis of a malignant solid tumour refractory to conventional chemotherapy or for whom no effective therapy existed were eligible. Other eligibility criteria included the following: age $\geqslant 18$ years; Eastern Cooperative Oncology Group performance status $\leqslant 1$; no previous anticancer therapy for at least 4 weeks (6 weeks for nitrosourea or mitomycin-C); and adequate haematopoietic (absolute neutrophil count (ANC) $\geqslant 1.5\times10^9$ cells/l, platelet count $\geqslant 100\times10^9$ cells/l and haemoglobin $\geqslant 100$ g/l (or 6.2 mmol/l), hepatic (serum total bilirubin $\leqslant 25.65$ μmol/l, and serum aspartate transaminase (AST), alanine transaminase (ALT) $\leqslant 2.5$ times the institutional upper normal limit (UNL) ($\leqslant 5.0$ times UNL in case of liver metastases), and renal function (serum creatinine concentration $\leqslant 132.6$ μmol/l). Prior surgery or radiation therapy (irradiation field encompassing $<25\%$ of bone marrow) was acceptable as long as it had been completed at least 4 weeks before study registration. Specific exclusion criteria included known hypersensitivity to Cremophor EL and/or paclitaxel-containing regimens, known brain metastases, spinal cord compression, and/or carcinomatous meningitis. The study protocol was approved by the institutional Ethical Board, and all patients gave written informed consent before study entry.

2.2. Treatment and dose escalation

LEP was provided in vials containing 25 mg of paclitaxel per vial, and was supplied by Pharmacia (Nerviano, Italy) as a freeze-dried product. The vials also contained cardiolipin, egg phosphatidyl choline, cholesterol, D-α-tocopheryl acid succinate (vitamin E), and mannitol as inactive ingredients. The addition of mannitol as a cryoprotectant to this liposomal formulation of paclitaxel ensured that sonication before the administration of the drug was not required. The vials were stored at 5 °C in the dark, and were kept at room temperature for at least 2 h before reconstitution. After that, 25 ml of 0.9% sodium chloride injection per 25 mg of paclitaxel were added to the LEP vials. The solution was injected in the middle of the lyophilised cake using a 50 ml sterile and pyrogen-free syringe. The vials were gently shaken for 2–3 minutes. The reconstituted product was a sterile dispersion, and in-line filters were not used for administration. The content of the reconstituted vials was transferred to an infusion bag using a syringe, and the infusion bag was gently turned for 30 seconds before infusion.

LEP was given as a 45-min infusion, preceded by premedication consisting of 20 mg dexamethasone, 2 mg clemastine, and 50 mg ranitidine, each administered intravenously (i.v.) 30 min before the initiation of LEP infusion. Prophylactic anti-emetics were not given. Treatment was administered every 7 days for 6 out of 8 weeks, unless the patient did not recover adequately from treatment-related adverse events of the prior infusions. A period of 8 weeks was defined as one cycle. The

starting dose of LEP was 90 mg/m^2/week. This dose and schedule was selected on the basis of both clinical and pharmacokinetic data regarding weekly paclitaxel and from a phase I study of a sonicated preparation of LEP given as a single agent once every 3 weeks (unpublished data, Pharmacia). Subsequent dose levels scheduled were: 120 mg/m^2/week (33% dose increment), 150 mg/m^2/week (25%), and 180 mg/m^2/week (20%).

At least three patients were entered at each dose level. The MTD was defined as one dose level below the dose that induced DLTs during the first cycle in $\geqslant 2$ out of 6 patients. DLTs were defined using the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2.0 and included: grade 4 neutropenia >7 days, grade 4 haematological toxicity of any duration (except for grade 4 neutropenia), febrile neutropenia, non-haematological toxicities $\geqslant$grade 3, severe hypersensitivity reaction suggestive of an anaphylactic reaction, and receiving less than 70% of the intended cumulative dose of LEP [17]. If grade 2 neutropenia and/or grade 2 thrombocytopenia occurred during treatment, the dose of LEP was decreased by 50% for the subsequent administration. In case of grade $\geqslant 3$ neutropenia and/or thrombocytopenia, treatment with LEP was omitted for that week and then decreased by 50% for subsequent administrations when the neutrophil count had recovered to $\geqslant 1.5\times10^9$ cells/l and the platelet count to $\geqslant 75\times10^9$ cells/l. Intrapatient dose escalation was not allowed.

2.3. Treatment assessment

Before initiating therapy, a complete medical history was taken and a physical examination was performed. A complete blood cell (CBC) count, including haematology tests (haemoglobin, white blood cells (WBC) with differential count, and platelets), and serum biochemistry, (sodium, potassium, calcium, magnesium, chloride, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, and gamma-glutamyltransferase) were performed, as were urinalysis (pH and albumin), electrocardiogram (ECG), and chest X-ray. Weekly evaluations included physical examination, toxicity assessment according to the NCI-CTC criteria, and haematology tests. Urinalysis was performed at week 1, 3 and 5 before the administration of LEP. Tumour evaluation was performed after every cycle of 8 weeks and response was assessed according to the Response Evaluation Criteria in Solid Tumours (RECIST) [18]. Treatment was discontinued in cases of disease progression, unacceptable toxicity or patient request.

2.4. Sample collection and drug analysis

The pharmacokinetics of LEP were evaluated by following the time-concentration profile of paclitaxel, measured both as total paclitaxel in blood (liposome-associated plus non-liposome-associated) and extraliposomal paclitaxel in plasma (non-liposome-associated, bound and unbound to plasma proteins). The pharmacokinetics of extraliposomal and total paclitaxel after LEP administration were evaluated in all patients enrolled in the study during the first cycle of treatment. A total of 33 blood samples (approximately 7 ml) were drawn from each patient during the first and last week of the first cycle of treatment at pre-dose, end of infusion and at 5, 15, 30 min and 1, 2, 4 h, any time between 8 and 16 h, 24, 48, 72 and 168 h (this latter only at the sixth week) post-infusion. In addition, blood samples were also collected at pre-dose and the end of infusion at the second, third, fourth, and fifth weeks of treatment. Blood samples were collected in precooled (ice-water, 4 °C) vials containing lithium heparin as the anticoagulant. An aliquot of blood (2 ml) was frozen at −20 °C and used for the analysis of total paclitaxel; the remaining amount of blood was centrifuged (1200 *g* for 15 min at 4 °C) and the harvested plasma was frozen at −20 °C and used for the analysis of extraliposomal paclitaxel.

Concentrations of total paclitaxel (i.e. the sum of liposome-associated and non-liposome-associated paclitaxel) and extraliposomal paclitaxel (i.e. the non-liposome-associated, protein-bound and unbound) were determined in blood and plasma, respectively, with validated methods based on liquid chromatography with tandem mass-spectrometric detection (MS/MS). For the quantitation of total paclitaxel, Triton X-100 (5%, (v/v)) was added to whole blood and an aliquot (100 μl) of blood was extracted using *tert*-butyl methyl ether (MTBE). To determine extraliposomal concentrations of paclitaxel in human plasma (250 μl), liposomes were separated from plasma proteins using a solution containing dodecantungstophosphoric acid and magnesium chloride; an aliquot of supernatant (100 μl) was extracted with MTBE. For both methods, paclitaxel was separated using a Zorbax C18 column and eluted under gradient conditions with a mobile phase containing acetonitrile and 2 mM ammonium acetate buffer (pH 5). MS/MS detection was conducted with a PE-Sciex API 3000 mass spectrometer using a turbo ionspray source and multiple reaction monitoring in a positive ion mode. The lower limits of quantitation were 5 ng/ml and 1 ng/ml for total paclitaxel in blood and extraliposomal in plasma, respectively.

Analysis of the protein-unbound fraction of the extraliposomal paclitaxel concentrations was attempted, but could not be determined separately due to interference in the equilibrium dialysis method [19].

2.5. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated by standard non-compartmental methods using the WinNonlin

version 3.1 (Pharsight, Mountain View, CA, USA). The area under the plasma concentration-time curve (AUC) was calculated up to the last detectable concentration ($C_{(t,z)}$), using the linear trapezoidal rule. Other parameters, including volume of distribution at steady state (V_{ss}) and clearance (CL) were estimated using standard equations. The AUC was extrapolated to infinity by the addition of $C_{(t,z)}/k$, where k is the terminal rate constant, which was estimated from a log-linear regression analysis of the terminal disposition phase. The half-life of the terminal phase was calculated as ln(2)/k.

3. Results

A total of 14 patients were entered onto the study and received at least one dose of LEP. All patients were assessable for toxicity and response. Patients' characteristics are listed in Table 1. Four patients did not complete the first cycle; one patient with adenocarcinoma of the duodenum, developed tumour-related haematemesis and three patients had disease progression. The total number of assessable cycles was 16. The median number of cycles per patient was 1 (range, 1–2).

Table 1
Patients' characteristics

Characteristic	No. of patients
No. of patients	
Total	14
Assessment	
For dose-limiting toxicity	13
For efficacy	12
No. of cycles/patient	
Median (range)	1 (1–2)
Gender	
Male	8
Female	6
Age (years)	
Median (range)	54 (30–66)
ECOG performance status	
0	4
1	10
Previous therapy	
Chemotherapy only	11
Radiotherapy	3
Surgery	9
Tumour types	
Colorectal	3
Gastro-intestinal tract, including:	8
Esophageal	1
Gastric	2
Gallbladder	1
Pancreatic	2
Unknown primary tumour	2
Miscellaneous	3

ECOG, Eastern Cooperative Oncology Group.

3.1. Dose-limiting toxicity

At 150 mg/m^2/week, one patient experienced DLT by receiving less than 70% of the intended cumulative dose of LEP. The patient received the first two doses as planned. The third and fourth infusions were omitted because of grade 3 and grade 4 neutropenia, respectively, and the patient received no subsequent therapy because of disease progression. In view of this single DLT, the cohort was expanded without observing further DLTs. Dose escalation to 180 mg/m^2/week did not take place because of discontinuation of the study (see below). For this reason, the MTD and RD were not determined.

3.2. Haematological toxicity

Haematological toxicities per patient over the entire course of treatment are summarised in Table 2. One patient experienced grade 3 neutropenia at the 120 mg/m^2/week dose level and one patient at the 150 mg/m^2/week dose level experienced grade 3 and grade 4 neutropenia. At the 150 mg/m^2/week dose level, haematological side-effects generally lasted less than 7 days. Mild to moderate anaemia and thrombocytopenia were documented at all dose levels tested.

3.3. Non-haematological toxicity

Gastrointestinal toxicities of mild to moderate severity were observed at all dose levels tested, and consisted of nausea [grade 1 ($N=7$), grade 2 ($N=1$), grade 3 ($N=1$)], diarrhoea [grade 1 ($N=8$), grade 2 ($N=1$), grade 3 ($N=1$)], and vomiting [grade 1 ($N=3$)]. Mild hypersensitivity reactions, consisting mainly of a facial flush and shortness of breath, were documented in four patients at the 150 mg/m^2/week dose level. All of these reactions had a rapid onset within the first minutes after the start of the LEP infusion and promptly recovered after stopping of the infusion and i.v. administration of 2 mg clemastine and 100 mg hydrocortisone. After rechallenging, none of these patients experienced a repeat of the infusion reaction. In two patients, a transient grade 1 to 2 skin reaction was documented. Mild alopecia (grade 1) was observed in 1 patient and neurotoxicity was not observed, but particularly the latter should be interpreted with caution in view of the very small number of cycles evaluated per patient.

3.4. Pharmacokinetics

Pharmacokinetic analysis was performed on all 14 patients enrolled in this study. The pharmacokinetic parameters for total and extraliposomal paclitaxel during the first and sixth week of the LEP treatment are summarised in Tables 3 and 4. After the administration

of LEP at dose levels 90, 120, and 150 mg/m^2/week, the levels of total and extraliposomal paclitaxel reached the maximum value near the end of infusion on both the first and sixth week of treatment. After the administration of 90, 120 and 150 mg/m^2/week, the mean (±S.D.) peak concentration of extraliposomal paclitaxel was 190±94 and 186±70 ng/ml, 363±241 and 224 ng/ml, and 424±166 and 326±120 ng/ml, after the first and sixth week, respectively. The corresponding values of total paclitaxel were 2787±1262 and 3083±807 ng/ml, 3918±1325 ng/ml and 2020 ng/ml, and 5004±2334 and 5032±3527 ng/ml, after the first and sixth week, respectively, at the 90, 120 and 150 mg/m^2/week dose levels. After the end of infusion, blood levels of total paclitaxel and plasma levels of extraliposomal paclitaxel declined polyexponentially with an apparent terminal half-life ranging between 77 and 195 h, and 80 and 144 h, respectively. Total paclitaxel exhibited a relatively slow clearance from whole blood (range, 9 to 26 l/h/m^2), with a steady-state volume of distribution ranging from 120 to 2189 l/m^2. There was large interpatient variation in both drug clearance and volume of distribution at a coefficient of variation of approximately 60%.

After the first week of treatment, the levels of total and extraliposomal paclitaxel increased in direct proportion with the dose, suggesting linear pharmacokinetics. Furthermore, over the tested dose range, total blood clearance was dose-independent ($P=0.490$,

Table 2
Haematological toxicity (worst grade per patient (pt))

			Grades										
			Anaemia		Leucocytopenia			Neutropenia			Thrombocytopenia		
Dose (mg/m^2/week)	No. of pts.	No. of cycles	1–2	3–4	1–2	3	4	1–2	3	4	1–2	3	4
90	3	3	3	0	2	0	0	1	0	0	1	0	0
120	4	4	2	0	2	0	0	1	1	0	2	0	0
150	7	9	5	0	3	3	0	3	1	1	2	0	0

Table 3
Mean±S.D. plasma pharmacokinetic parameters of total paclitaxel during the 1st week and 6th week of LEP treatment

Dose (mg/m^2/week)	No. of patients	1st week						6th week			
		C_{max} (ng/ml)	$T_{1/2,z}$ (h)	$AUC_{0-t(last)}$ (ng·h/ml)	$AUC_{0-\infty}$ (ng·h/ml)	CL (l/h/m^2)	V_{ss} (l/m^2)	No. of patients	C_{max} (ng/ml)	$T_{1/2,z}$ (h)	$AUC_{0-t(last)}$ (ng·h/ml)
90	3	2787±1262	135±67	6444±12	8035±1111	11±2	1004±506	3	3083±807	195[a]±61	12 494±5194
120	4	3918±1325	77±68[a]	6566±3231	7611±2492	17±5	618±316	1	2020	112	5731
150	7	5004±2334	118±83	10 609±2852	12 539±3260	13±6	1036±824	5	5032±3527	145[b]±125	13 759±5143

C_{max}, peak blood concentration; $T_{1/2,z}$, half life of the terminal disposition phase; $AUC_{0-t(last)}$, area under the blood concentration-time curve up to the last time point with measurable levels; $AUC_{0-\infty}$, AUC extrapolated to infinity; CL, total blood clearance; V_{ss}, steady-state volume of distribution.

[a] [a]$N=2$.

[b] $N=3$.

Table 4
Mean±SD plasma pharmacokinetic parameters of extraliposomal (protein-bound) paclitaxel during the 1st week and 6th week of LEP treatment

Dose (mg/m^2/week)	No. of patients	1st week				6th week			
		C_{max} (ng/ml)	$T_{1/2,z}$ (h)	$AUC_{0-t(last)}$ (ng·h/ml)	$AUC_{0-\infty}$ (ng·h/ml)	No. of patients	C_{max} (ng/ml)	$T_{1/2,z}$ (h)	$AUC_{0-t\ (last)}$ (ng·h/ml)
90	3	190±94	80[a]	910±171	N/A	3	186±70	135±67	4242±2658
120	4	363±241	138[b]±74	1357±287	2119±576	1	224[a]	N/A	2826
150	7	424±166	126[b]±32	2410±704	3031±839	5	326±120	144[c]±48	3967±1321

C_{max}, peak blood concentration; $T_{1/2,z}$, half life of the terminal disposition phase; $AUC_{0-t(last)}$, area under the blood concentration-time curve up to the last time point with measurable levels; $AUC_{0-\infty}$, AUC extrapolated to infinity; N/A not available.

[a] $N=1$.

[b] $N=3$.

[c] $N=2$.

Kruskal–Wallis one-way ANOVA, corrected for ties), supporting the above observation of a linear kinetics for total paclitaxel. Pharmacokinetic information obtained during the sixth week indicated on average an one- to two-fold accumulation, which is in reasonable agreement with the half-life of the apparent terminal disposition phase.

Assuming an equal distribution between plasma and blood for extraliposomal paclitaxel following the administration of LEP [20], extraliposomal paclitaxel represented only a minor portion of the total paclitaxel (i.e. the sum of liposome-associated and non-liposome-associated paclitaxel) in the systemic circulation. The proportion of extraliposomal and total paclitaxel changed with time and among patients, ranging from 3 to 14% of the total paclitaxel at the first time point to approximately 23–100% at the final sampling time points. On average, considering the overall exposure, extraliposomal paclitaxel in the plasma accounted for approximately 14–49% of the total paclitaxel exposure. The mean extraliposomal paclitaxel plasma concentrations and the total paclitaxel concentrations in blood versus time curves observed at the 150 mg/m^2/week dose level are displayed in Figs. 1 and 2.

3.5. Antitumour efficacy

At the 150 mg/m^2/week dose level, disease stabilisation for 8 weeks was documented in two patients with liver metastases of an adenocarcinoma of unknown primary and with pleural and peritoneal metastatic oesophageal carcinoma, respectively. The other patients had progressive disease after the tumour assessment at 8 weeks, with the exception of 2 patients who discontinued treatment because of early progression after the first and fourth administrations of LEP, respectively.

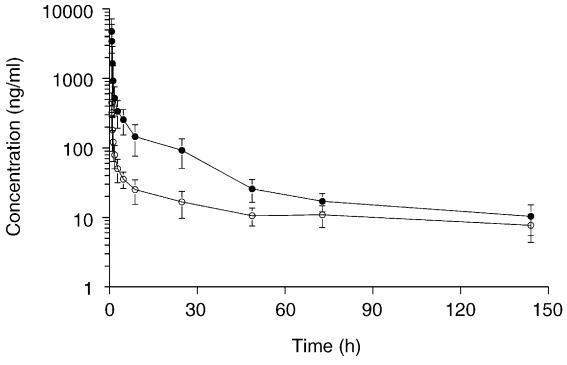

Fig. 1. Plasma concentration-time profiles of total paclitaxel in whole blood (closed symbols) and extraliposomal paclitaxel in plasma (open symbols) in patients receiving liposome encapsulated paclitaxel (LEP) at a dose of 150 mg/m^2/week. Data are presented as mean values (symbol)±standard deviation (S.D.).

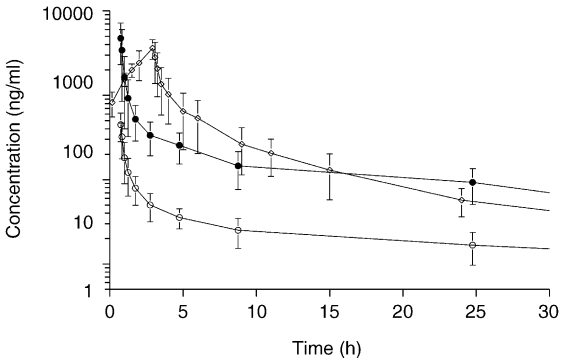

Fig. 2. Plasma concentration-time profiles of total paclitaxel in whole blood (closed symbols), extraliposomal paclitaxel in plasma (open symbols) in patients receiving liposome encapsulated paclitaxel LEP at a dose of 150 mg/m^2/week, and total paclitaxel in plasma (open lozenges) in 14 patients receiving Taxol® at a dose of 150 mg/m^2 (3-h infusion). Data are presented as mean values (symbol)±standard deviation (S.D.).

4. Discussion

The current study was performed to explore the safety, feasibility, and pharmacokinetics of a new liposomal formulation of paclitaxel administrated as a 45 min i.v. infusion once weekly for 6 out of 8 consecutive weeks. A previous formulation requiring a cumbersome sonication step just prior to infusion had been tested in a phase I trial using a single dose given every 21 days. The new, freeze-dried formulation has the same composition as the previously tested LEP formulation with the exception of the addition of mannitol as a cryoprotectant. The non-sonicated formulation was developed with the objectives of improving the overall profile of the drug. Real-time pharmacokinetics complimented the clinical findings indicating that LEP was unlikely to offer significant advantages over the taxanes currently employed in the clinic. With the new formulation, myelosuppression was seen at the 150 mg/m^2/week dose level, suggesting that a possible recommended phase II dose using a weekly schedule would be in the range of 150–180 mg/m^2/week. However, the current results do not allow any conclusions regarding long-term or cumulative toxicity, including neurotoxicity.

To be effective as a carrier, a liposome must be able to efficiently balance stability in the circulation with the ability to make the drug available at the tumour [8]. In order to achieve the optimum efficacy for a drug-delivery system, it is necessary to encapsulate the maximum possible quantity of a drug [7]. Comparison of the encapsulation efficiency of the drug in liposomes with the therapeutic dose indicates whether liposomes can be used as a suitable drug-delivery system [7]. The retention of the encapsulated drug is determined by the physicochemical characteristics of the drug itself and by the lipid composition and number of concentric membranes

of the liposomal vesicle [21,22]. Highly hydrophobic drugs, like paclitaxel, tend to associate mainly with the bilayer compartment of the liposome, resulting in lower entrapment stability due to faster redistribution of the drug to plasma components [9].

In comparison with small molecules, the volume of distribution of the drug encapsulated in liposomes is usually significantly reduced [9], and when a drug is stably encapsulated within the liposomal matrix it displays the pharmacokinetic profile of the intact liposome rather than that of the encapsulated agent [11]. In general, this should achieve a significant increase in the AUC in the circulation, and possibly in tumour tissue, and mimick the effect of administering cytotoxic drugs as a continuous i.v.infusion, without the inconvience of i.v. devices and toxicities associated with systemic drug exposure [11]. Likewise, the clearance of anticancer drugs encapsulated in liposomes is usually reduced, and the elimination half-life prolonged [23], as has been shown previously for anthracyclines [24], vincristine [25] and lurtotecan [26].

The pharmacokinetics of total paclitaxel when administered as LEP appeared to be dose-independent, providing further evidence of the previous supposition that the non-linearity of paclitaxel disposition following the administration of Taxol® is caused by its excipient [1]. However, as compared with Taxol®, the interpatient variation in paclitaxel pharmacokinetic parameters following the administration of LEP was large (up to 60%). In contrast to that expected for a liposomal formulation, the clearance of total paclitaxel in whole blood following LEP (15.3 ± 8.98 l/h/m^2) was very similar to that reported after administration of Taxol® (17.5 ± 3.43 l/h/m^2) [27]. The observed values for volume of distribution at steady state of total paclitaxel in patients receiving LEP was very high (approximately 1000 l/m^2). Furthermore, in the systemic circulation, most paclitaxel was associated with the liposomes, since the extraliposomal paclitaxel AUC accounted only for 14–49% of the total paclitaxel AUC; however, the proportion of extraliposomal drug in plasma and total drug in whole blood increased with the time, reaching unity at the end of the sampling time period.

Previous work has shown that the plasma protein binding of the fraction of unbound paclitaxel in plasma in the absence of formulation excipients is approximately 85% in humans [1]. Assuming this value of plasma protein binding also for paclitaxel after LEP administration, at the 150 mg/m^2/week dose level, the predicted $AUC_{0-t(last)}$ is 362 ± 106 ng·h/ml, which is similar to 397 ± 69.7 ng·h/ml observed for Taxol® at the recommended weekly dose of 100 mg/m^2 [28]. This clearly suggests that at approximate equitoxic doses, exposure to the clinical relevant pharmacokinetic parameter is comparable for both formulations, and that LEP provides no pharmacological advantages over Taxol®.

The release of complement fragments C3a and C5a can cause a hypersensitivity reaction, called complement activation-related pseudoallergy (CARPA), by release of anaphylatoxins and a cascade of cellular mediators of inflammation [3,29,30]. Activation of complement can rapidly induce several symptoms, including pain (e.g. chest-pain, low back pain, headache), chills, choking, nausea, confusion, skin toxicity (e.g. erythema, pruritus, urticaria), symptoms of respiratory distress (e.g. bronchospasm, dyspnoea), and severe cardiac arrhythmias [3,29]. The frequency of CARPA due to i.v. infusion of conventional or pegylated liposomes ranged from 3 to 7% in several studies. Symptoms were observed within 5 to 10 min after the start of the first infusion. In most patients, symptoms disappeared shortly after stopping of the infusion [3]. The rapid induction of this event seems to indicate that minimal amounts of liposomes can induce these side-effects [30]. In the present study, hypersensitivity reactions were observed shortly after the i.v. infusion of the first LEP treatment in 3 patients. Rechallenge of the LEP infusion after treatment of corticosteroids and antihistamines was possible in all 3 patients without any new reaction or other complications. While the frequency of reactions was relatively low, LEP did not distinguish itself from currently used taxanes, since all patients received standard premedication with dexamethasone and antihistamines.

Collectively, the results of pharmacokinetic data supported the decision to terminate the study prior to reaching the primary objective of determining the MTD and RD and strengthened the importance of performing a real-time pharmacokinetic analysis during a phase I study in order to bring as much as relevant information as possible to guide future clinical treatment decisions.

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