Distribution of paclitaxel in plasma and cerebrospinal fluid

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Our objective was to assess the distribution of paclitaxel in plasma and cerebrospinal fluid (CSF) in a cancer patient, and evaluate the role of the formulation vehicle Cremophor EL (CrEL) in drug distribution. Analysis of paclitaxel concentrations in CSF was performed using a triple-quadrupole mass spectrometric assay with electrospray ionization. Total and unbound paclitaxel levels in plasma were measured by liquid chromatography and equilibrium dialysis, respectively, and CrEL concentrations were determined by a colorimetric dye-binding microassay. Clinical samples were obtained from a 54-year-old female with breast cancer receiving a weekly regimen of paclitaxel (dose 60 mg/m^2). The disposition of total paclitaxel in plasma was characterized by a bi-exponential elimination (terminal half-life 9.17 h) and a total clearance of 19.4 l/h/m^2. The fraction of unbound paclitaxel in plasma ranged from 7.6 to 12.4% (unbound drug CL 176 l/h/m^2). The plasma clearance of CrEL was 0.332 l/h/m^2, whereas CrEL levels were undetectable in CSF (below 0.5 μl/ml). Concentrations of paclitaxel in CSF (range 45.5–162 pg/ml) and unbound CSF:unbound plasma concentration ratios (range 0.093–9.53%) progressively increased up to 24 h, with a mean unbound drug fraction in CSF of 84 ± 3.6% (range 81–88%). These findings indicate that there is substantial distribution of paclitaxel to CSF. Since the fraction of unbound paclitaxel is different between plasma and CSF, measurement of unbound paclitaxel is required to accurately assess the extent of drug penetration.
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Introduction

Paclitaxel is a highly lipophilic antineoplastic agent formulated for clinical use in a mixture of Cremophor EL (CrEL) and dehydrated ethanol [1]. CrEL has been proposed to cause the non-linear pharmacokinetic behavior of paclitaxel by trapping the drug in micelles, and making it less available for distribution to tissues, metabolism and biliary secretion [2]. It is generally acknowledged that the unbound drug fraction is capable of diffusing across biological barriers and to interact with target sites in the extravascular compartment, such as the cerebrospinal fluid (CSF) [3]. Since the unbound fraction of paclitaxel is the pharmacologically active form, understanding factors influencing paclitaxel penetration into CSF might be important in predicting antitumor activity in patients with leptomeningeal or intracranial malignant disease. In view of the high affinity of paclitaxel for both serum proteins and the hydrophobic interior of CrEL micelles [4,5], we speculated that the distribution of unbound paclitaxel to CSF is influenced by CrEL and may vary over time following drug administration. The aim of this study was to assess the extent of drug penetration into CSF in a cancer patient taking vascular binding of paclitaxel into consideration.

Materials and methods

Patient history and treatment

The studied patient was a 54-year-old female with advanced breast cancer who had received previous adjuvant therapy with FEC, radiation and tamoxifen (five courses), FAC (five courses), CMF (six courses), and whole brain radiotherapy because of leptomeningeal metastases. She also had non-insulin-dependent diabetes mellitus. Due to liver metastases, she had mild hepatic dysfunction [transaminases, National Cancer Institute Common Toxicity Criteria (NCI-CTC) grade 2; bilirubin, NCI-CTC grade 1 (16 μmol/l)] and it was decided to administer a reduced weekly paclitaxel dose of 60 mg/m^2 (absolute dose 95 mg; Bristol Myers Squibb, Woerden, The Netherlands). Tests for renal and bone marrow function were within normal limits. The drug was administered as a solution in 500 ml of 0.9% (v/v) isotonic sodium chloride and given via a peripheral catheter using a motor-driven programmable infusion pump (model 598;

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IVAC, San Diego, CA) over a 3-h period. Intravenous premedication consisted of dexamethasone (20 mg), clemastine (2 mg) and cimethidine (300 mg). Additional co-medication included gliclazide (80 mg, 3 times a day), pantoprazole (40 mg), temazepam (20 mg) and tolbutamide (500 mg, 3 times a day). The protocol was approved by the Erasmus MC review board (Rotterdam, The Netherlands) and the patient provided written informed consent.

Sample collection

Blood samples were collected at the following time points: immediately before infusion; at 1.13 and 2.00 h after the start of infusion; 5 min before the end of infusion (2.92 h); and 11, 25, 35 min, and 1.13, 2.15, 4.13, 7.08, 9.13 and 21.33 h after the end of infusion. Blood was collected in 10 ml tubes containing potassium-EDTA as anti-coagulant. Plasma was separated by centrifugation (3000 *g* for 10 min at 4°C), aliquotted in 1.5-ml fractions and stored at −20°C until analysis. CSF samples of 5 ml each were obtained at 3.37, 5.10 and 24.13 h after the start of infusion from repeated lumbar punctures, and immediately stored at −20°C. These samples were also analyzed for total protein content and number of contaminating erythrocytes.

Analytical assays

Plasma concentrations of total paclitaxel, unbound paclitaxel and CrEL were determined as described elsewhere [6–8]. The assay for paclitaxel in CSF was based on previous procedures for docetaxel and its vehicle polysorbate 80 [9,10]. In brief, a one-step extraction was performed from sample aliquots with 7 ml of acetonitrile:*n*-butyl chloride (1:4, v/v). Chromatographic analyses were performed using a Waters Alliance LC system (Milford, MA) and a Waters X-Terra column (20 × 2.1 mm, internal diameter) packed with a 3.5-μm RP_{18} stationary phase. The mobile phase was composed of methanol:water (85:15, v/v) containing 0.1% formic acid (flow rate 0.15 ml/min). The column effluent was monitored using a Micromass Quattro LC triple-quadrupole mass spectrometric detector with an electrospray interface and controlled by the MassLynx version 3.4 software (Micromass, Beverly, MA). The samples were analyzed using an electrospray probe in the positive ionization mode operating at a collision energy of 20 eV and a cone voltage of 30 V. Samples were introduced into the interface through a heated nebulizer probe (350°C). The spectrometer was programmed to allow the $[M\text{-}H]^+$ ion of paclitaxel (*m/z* 854.5) and that of the internal standard docetaxel (*m/z* 808.6) to pass through the first quadrupole and into the collision cell. The daughter ions for paclitaxel (*m/z* 286.1) and docetaxel (*m/z* 527.1) were monitored through the third quadrupole. Argon was used as collision gas at a pressure of 0.0027 mbar and the dwell time per channel was 0.5 s for data collection.

The retention time for paclitaxel was 1.56 ± 0.20 min with an overall chromatographic run time of 5.0 min. Calibration curves were constructed in Elliott's B solution (range 0.1–1.0 nM), and computed using the peak area ratio of paclitaxel and docetaxel by weighted ($1/x$) linear-regression analysis ($R > 0.991$). Deviations from the interpolated concentrations were all within the acceptable 80–120% of the nominal values (range 90.1–110.2%). Accuracy values for quality-control samples, analyzed in duplicate at 0.3 and 0.7 nM, were between 87.3 and 105.1%. The lower limit of quantitation of paclitaxel in CSF was 0.1 nM (85.4 pg/ml; signal-to-noise ratio = 3), using 1-ml samples.

Pharmacological analysis

Plasma concentration–time profiles of paclitaxel were analyzed by a two-compartment model using Siphar version 4.0. (InnaPhase, Philadelphia, PA) [5]. Estimates of pharmacokinetic parameters for CrEL were derived from non-compartmental analysis using WinNonLin version 3.0 (Pharsight, Mountain View, CA) [11]. CSF:plasma concentration ratios were calculated using the simulated (model-estimated) plasma concentrations at the time point of CSF sampling.

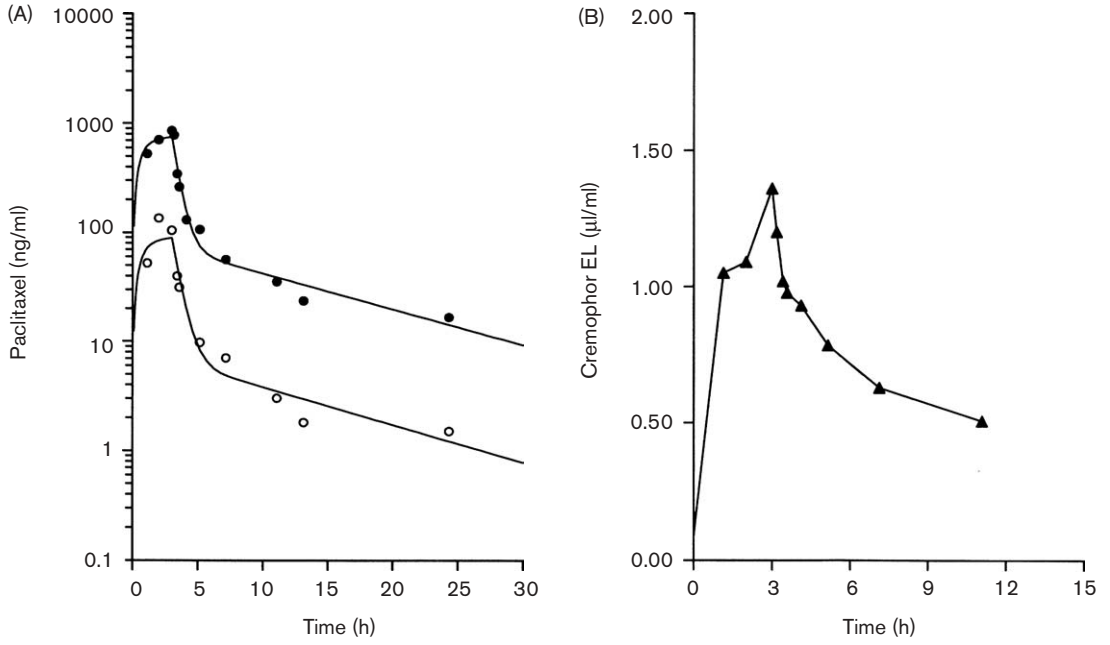
Results and discussion

This study was performed to assess the extent of paclitaxel distribution to CSF in a cancer patient with breast cancer and leptomeningeal metastases. The patient's body surface area was 1.60 m^2 and she received a dose of 95 mg paclitaxel using a 3-h infusion. Overall, the treatment was very well tolerated, without any sign of substantial hematological or central nervous system toxicity.

The disposition of total paclitaxel in plasma was characterized by a bi-exponential elimination (terminal half-life 9.17 h) (Fig. 1), with the fraction of unbound paclitaxel in plasma ranging from 7.6 to 12.4%. Total and unbound paclitaxel clearances were 19.4 and 176 l/h/m^2 (Table 1), respectively, in line with previous findings obtained in 14 patients receiving a dose of 100 mg/m^2 [mean total drug (range) 21.8 ± 8.41 (9.13–39.2) l/h/m^2; mean unbound drug (range) 123 ± 61.0 (61.0–257) l/h/m^2] [5]. This suggests that the mild liver function impairment in this patient had no evident effect on paclitaxel clearance. The CrEL clearance of 0.332 l/h/m^2 (Table 1) was also similar to earlier observations [mean (range) 0.207 ± 0.127 (0.085–0.571) l/h/m^2] [5].

CSF samples did not contain residual blood (1×10^6 erythrocytes or lower) and the mean total protein concentration was 0.69 g/l. Given the low protein concentration relative to that observed in plasma (range 38–45 g/l), a correction for binding to proteins as well as CrEL was performed by measuring total and unbound

Fig. 1



Plasma concentration–time curves of total paclitaxel (closed symbols) and unbound (open symbols) paclitaxel (A) and CrEL (B) during and after a 3-h infusion of paclitaxel at a dose of 60 mg/m^2. The mathematical equations describing the paclitaxel concentration ($C_{(t)}$) at any time (t) during (eq. 1) and after i.v. administration (eq. 2) are given by: $C_{(t)} = \Sigma C_i/(\lambda_i \times T_{inf}) \times (1 - e^{(-\lambda i \times t)})$ (eq. 1) and $C_{(t)} = \Sigma C_i/(\lambda_i \times T_{inf}) \times (e^{(-\lambda i \times [t - Tinf])} - e^{(-\lambda i \times t)})$ (eq. 2). The model parameters were $C_1 = 3.663$ µg/ml, $C_2 = 0.079$ µg/ml, $\lambda_1 = 1.797\ h^{-1}$ and $\lambda_2 = 0.0756\ h^{-1}$ for total paclitaxel ($R^2 = 0.977$), and $C_1 = 0.394$ µM, $C_2 = 0.0074$ µM, $\lambda_1 = 1.585\ h^{-1}$ and $\lambda_2 = 0.0792\ h^{-1}$ for unbound paclitaxel ($R^2 = 0.963$).

Table 1 **Summary of pharmacokinetic parameters**

Parameter	C_p	C_u	CrEL
Plasma			
C_{max} (µg/ml)	0.857	0.136	1.36[a]
AUC (µg·h/ml)	3.09	0.342	15.0[b]
CL (l/h/m^2)	19.4	176	0.332
V_d (l/m^2)	257	2215	4.28
$T_{1/2,\ z}$ (h)	9.17	8.75	8.92
MRT (h)	4.87	3.90	11.5
CSF			
$C_{3.37\,h}$ (pg/ml)	45.5	36.9	NQ
$C_{5.10\,h}$ (pg/ml)	114	94.6	NQ
$C_{24.13\,h}$ (pg/ml)	162	143	NQ

C_p, total paclitaxel; C_u, unbound paclitaxel; C_{max}, peak plasma concentration; AUC, area under the plasma concentration–time curve; CL, systemic clearance; V_d, volume of distribution; $T_{1/2,\ z}$, half-life of the terminal disposition phase; MRT, mean residence time; C_{ih}, concentration in sample taken at i h after start of paclitaxel infusion; NQ, not quantifiable.
[a]Data expressed in µl/ml.
[b]Data expressed in µl·h/ml.

paclitaxel concentrations in CSF. The free drug fraction of paclitaxel was relatively constant and averaged 84 ± 3.6% (range 81–88%). CrEL levels were undetectable in CSF (i.e. below 0.5 µl/ml). Concentrations of paclitaxel in CSF were less than those in plasma at all sampled time points and apparent equilibrium was not attained within the 24-h period (Table 1). The CSF:plasma unbound concentration ratios progressively increased from 0.093% at 3.3 h to 0.98% at 5.10 h and 9.53% at 24 h after drug administration. The limited surface area for paclitaxel diffusion and the hydrophobic nature of the drug with extensive vascular binding to serum proteins and CrEL likely contributed to the slow equilibrium kinetics. Overall, CSF represented a small additional compartment for paclitaxel distribution, particularly in view of the large volume of distribution of unbound paclitaxel in this patient of 2215 l/m^2.

In a previous trial, paclitaxel could not be detected in CSF of leukemic patients by high-performance liquid chromatography with a lower limit of quantitation of 50 nM [12]. Using more sensitive analytical procedures based on liquid chromatography or radioimmunoassay, paclitaxel has been detected more recently in CSF of several patients receiving paclitaxel at doses of 90–315 mg/m^2 [13–15]. This current investigation adds to that knowledge because (i) it is the first to take into account the vascular binding of paclitaxel to proteins and CrEL by measuring unbound paclitaxel concentrations, and (ii) it reports on the feasibility of measuring drug levels after low paclitaxel doses following weekly

regimens using a highly sensitive assay based on mass-spectrometric detection. It should also be noted that, because the CSF:plasma unbound concentration ratios are by no means constant parameters during the dosing interval, single-point data are clearly inappropriate to directly estimate the extent of penetration by paclitaxel, as previously noted [15]. Although the described data on paclitaxel accumulation are limited to only one patient, the results suggest that i.v. paclitaxel administration at low doses can produce adequate drug distribution to CSF at concentrations associated with significant antitumor activity in experimental models [16]. The paclitaxel penetration and subsequent accumulation in CSF thus might offer a potential therapeutic advantage in that tumor cells in the central nervous system are exposed to relatively high local unbound drug levels for prolonged time periods.

References

1 Gelderblom H, Verweij J, Nooter K, Sparreboom A. Cremophor EL: the drawbacks and advantages of vehicle selection for drug formulation. *Eur J Cancer* 2001; **37**:1590–1598.

2 Henningsson A, Karlsson MO, Vigano L, Gianni L, Verweij J, Sparreboom A. Mechanism-based pharmacokinetic model for paclitaxel. *J Clin Oncol* 2001; **19**:4065–4073.

3 Barre J, Didey F, Delion F, Tillement JP. Problems in therapeutic drug monitoring: free drug level monitoring. *Ther Drug Monit* 1988; **10**:133–143.

4 Sparreboom A, van Zuylen L, Brouwer E, Loos WJ, De Bruijn P, Gelderblom H, *et al.* Cremophor EL-mediated alteration of paclitaxel distribution in human blood: clinical pharmacokinetic implications. *Cancer Res* 1999; **59**:1454–1457.

5 Gelderblom H, Mross K, ten Tije AJ, Behringer D, Mielke S, Van Zomeren DM, *et al.* Comparative pharmacokinetics of unbound paclitaxel during 1- and 3-hour infusion. *J Clin Oncol* 2002; **20**:574–581.

6 Brouwer E, Verweij J, Hauns B, Loos WJ, Nooter K, Mross K, *et al.* Linearized colorimetric assay for Cremophor EL: application to pharmacokinetic after 1-hour paclitaxel infusions. *Anal Biochem* 1998; **261**:191–202.

7 Brouwer E, Verweij J, de Bruijn P, Loos WJ, Pillay M, Buijs D, *et al.* Measurement of fraction unbound paclitaxel in human plasma. *Drug Metab Dispos* 2000; **28**:1141–1145.

8 Sparreboom A, de Bruijn P, Nooter K, Loos WJ, Stoter G, Verweij J. Determination of paclitaxel in human plasma using single solvent extraction prior to isocratic reversed-phase high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B* 1998; **705**:159–164.

9 Baker SD, Zhao M, Zabelina Y, Donehower RC, Wolff AC. Rapid and sensitive method for the determination of docetaxel in human plasma using high-performance liquid chromatography-tandem mass spectrometry (LC-MS-MS). *Proc Am Ass Cancer Res* 2001; **42**:459 (abstr 2059).

10 Sparreboom A, Zhao M, Brahmer JR, Verweij J, Baker SD. Determination of the docetaxel vehicle, polysorbate 80, in patient samples by liquid chromatography–tandem mass spectrometry. *J Chromatogr B* 2002; **773**:183–190.

11 Sparreboom A, Wolff AC, Verweij J, Zabelina Y, Van Zomeren DM, McIntire GL, *et al.* Disposition of DHA-paclitaxel, a novel taxane, in blood: *in vitro* and clinical pharmacokinetic studies. *Clin Cancer Res* 2003; **9**:151–159.

12 Rowinsky EK, Burke PJ, Karp JE, Tucker RW, Ettinger DS, Donehower RC. Phase I and pharmacodynamic study of taxol in refractory acute leukemias. *Cancer Res* 1989; **49**:4640–4647.

13 Heimans JJ, Vermorken JB, Wolbers JG, Eeltink CM, Meijer OW, Taphoorn MJ, *et al.* Paclitaxel (Taxol) concentrations in brain tumor tissue. *Ann Oncol* 1994; **5**:951–953.

14 Glantz MJ, Choy H, Kearns C, Mills PC, Wahlberg LU, Zuhowski EG, *et al.* Paclitaxel disposition in plasma and central nervous systems of humans and rats with brain tumors. *J Natl Cancer Inst* 1995; **87**:1077–1081.

15 O'Boyle KP, Wang Y, Schwartz EL, Regl DL, Einzig A, Dutcher JP, *et al.* Development of two radioimmunoassays to detect paclitaxel in sera and in cerebrospinal, ascitic and pleural fluids. *Cancer* 1997; **79**:1022–1030.

16 Tseng SH, Bobola MS, Berger MS, Silber JR. Characterization of paclitaxel (Taxol) sensitivity in human glioma- and medulloblastoma-derived cell lines. *Neuro-Oncology* 1999; **1**:101–108.