Limited cerebrospinal fluid penetration of docetaxel

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Our purpose was to investigate the cerebrospinal fluid (CSF) penetration of docetaxel in cancer patients. Docetaxel was administered as a 1-h infusion at a dose of 75 mg/m^2 to two patients with metastatic breast cancer and leptomeningeal carcinomatosis. CSF samples were obtained using a lumbar puncture up to a 72-h time period. Total and unbound docetaxel concentrations in plasma and CSF were determined by liquid chromatography (lower limit of quantitation: 0.5 nM for plasma and 0.050 nM for CSF) and equilibrium dialysis, respectively. The pharmacokinetics of docetaxel in plasma are in line with data of previous studies. The concentrations of docetaxel in CSF did not follow the general pattern in plasma, with relatively stable concentrations over the 72-h time period. The fraction of unbound docetaxel in plasma ranged from 6 to 13%, while that in CSF ranged from 67 to 103%. For total and unbound docetaxel, the CSF to plasma concentration ratio progressively increased in 72 h from 0.01 to 0.6% and from 0.1 to 9%, respectively. These data suggest that measurement of unbound docetaxel is required to accurately assess the extent of drug penetration into CSF and that the drug can produce distribution to CSF at levels associated with significant antitumor activity in experimental models. *Anti-Cancer Drugs* **15:715–718**

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Introduction

Knowledge on the penetration of anticancer drugs into the central nervous system is essential for tumor targets localized within the brain. The pharmacodynamics of the drug in the central nervous system depends largely on the concentration–time profile at the site of action. This concentration–time profile is determined by several factors, including transport across the blood–brain barrier and the blood–cerebrospinal fluid (CSF) barrier [5]. For obvious reasons, direct measurement of drug concentrations in the brain tissue of cancer patients is highly restricted. Hence, in the clinical setting, drug concentrations in CSF are commonly used as a surrogate for drug concentrations in the brain [5]. However, the brain consists of multiple compartments and many factors, such as the presence of drug-transporting proteins and disruption of the blood–brain barrier by tumor cells, are involved in the process of altering the transport of drugs to these compartments. In the current study, CSF concentrations of docetaxel were used to evaluate the extent of drug delivery to the brain and, in particular, the meninges in two patients with metastatic breast cancer, because docetaxel is a commonly used drug in this malignancy [8], which is frequently associated with leptomeningeal carcinomatosis [4].

Patients and methods

Patients and treatment

Pharmacokinetic studies were performed on two patients treated for metastatic breast cancer with single-agent docetaxel. The patients received docetaxel (Taxotere; Aventis, Hoevelaken, The Netherlands) as a 1-h infusion at a dose of 75 mg/m^2 (absolute dose, 120 and 125 mg, respectively). Both patients had a WHO performance score < 2; normal kidney function (serum creatinine < 130 μmol/l); adequate hepatic function [total serum bilirubin $< 1.5 \times$ upper limit of institutional normal (ULN); transaminases < 2 times ULN; and alkaline phosphatase < 2 times ULN]; and adequate bone marrow function (absolute neutrophil count $> 1.5 \times 10^9$/l and platelet count $> 100 \times 10^9$/l). Both patients gave written informed consent and the Ethics Board of the Erasmus MC (Rotterdam, The Netherlands) approved the study.

Sample collection

Blood and CSF samples in the two patients were collected up to 25 and 72 h, respectively. Blood samples were drawn from a venous access site into heparinized tubes, separate from the site of the docetaxel infusion, while CSF samples were obtained by lumbar puncture.

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Blood samples were centrifuged immediately for 5 min at 2500 *g* to separate plasma, and CSF samples and plasma were stored at a temperature lower than –70°C in propylene vials, until analysis. Prior to analysis, it was confirmed that the CSF samples were not contaminated with blood (i.e. $< 1 \times 10^6$ erythrocytes), except for one sample, which was not taken into consideration in the final analysis.

Docetaxel analysis

Analytical measurement of total docetaxel concentrations in plasma was performed using a validated assay based on liquid chromatography with tandem mass spectrometric detection [lower limit of quantitation, 0.5 nM (around 0.4 ng/ml)], as described previously [1]. For determination of total docetaxel in CSF, the method was slightly modified. In brief, aliquots of 1 ml were extracted using a mixture of acetonitrile and *n*-butyl chloride (1:4, v/v) following the addition of the internal standard, paclitaxel. Chromatographic separations were achieved on a Waters X-Terra MS column (20 × 2.1 mm internal diameter) packed with a 3.5-μm octadecyl stationary phase (Waters), and a mobile phase composed of acetonitrile and 0.1% aqueous formic acid (80:20, v/v) that was delivered at a flow rate of 0.15 ml/min. Sample extracts were analyzed using a Micromass Quattro LC triple-quadrupole mass spectrometry detector (Beverly, MA) with an electrospray probe in the positive ionization mode. The spectrometer was programmed to detect the protonated molecular ion/product ion pairs of docetaxel (*m*/*z* 808.5, *m*/*z* 527.2) and paclitaxel (*m*/*z* 854.5, *m*/*z* 509.4). Calibration curves were constructed in Elliott's B solution over the range 0.050–1.0 nM, and computed using the peak area ratio of paclitaxel and docetaxel by weighted (1/*x*) linear regression analysis. The lower limit of quantitation of the assay for docetaxel in CSF is 0.050 nM (around 40 pg/ml).

For the determination of the fraction unbound docetaxel in plasma and CSF, a validated equilibrium dialysis method was used [9]. In brief, aliquots of 260 μl plasma or CSF were dialyzed against an equal volume of phosphate-buffered saline containing a [G-^{3}H]docetaxel tracer over a membrane with a 12 000–14 000 Da molecular weight cut-off (Spectrum Medical, Houston, TX). Dialysis experiments were performed using 2-ml polypropylene Safe-Lock vials (Eppendorf, Hamburg, Germany) as dialysis chambers in a humidified atmosphere at 37°C. After the end of the 48-h dialysis period, the radioactivity was measured by liquid scintillation counting for 20 min using a Wallac 1409 counter (Turku, Finland). The fraction of unbound docetaxel was expressed as a percentage, while the unbound docetaxel concentration was calculated as the product of the fraction of unbound docetaxel and the concentration of total docetaxel.

Pharmacokinetic analysis

Pharmacokinetic parameters estimates of docetaxel were derived from weighted (1/*y*) non-compartmental analysis using WinNonlin version 4.0 (Pharsight, Mountain View, CA). The CSF to plasma concentration ratios for docetaxel were calculated using the concurrent plasma concentrations at the time point of CSF sampling.

Results

A summary of the pharmacokinetics of total and unbound docetaxel in plasma and CSF is presented in Table 1. The values for total docetaxel parameters showed a 2.5-fold variation in clearance between the two patients, but are in line with data from several previous studies on docetaxel pharmacokinetics [3]. Using the applied analytical method, docetaxel concentrations in CSF could not be quantified in one patient, while the concentrations in the CSF from the other patient, in which the drug was cleared fast, were not quantifiable (i.e. levels were below 40 pg/ml), as were the concentrations in pre-dose CSF. The fraction unbound docetaxel in plasma ranged from 6 to 13% in samples from the two patients, while those in CSF ranged from 67 to 103%, presumably because of lower concentration of binding proteins in CSF compared to plasma. The concentration time curves of total and unbound docetaxel in plasma and CSF are presented in Figure 1. The concentrations of docetaxel in CSF did not follow the general pattern of docetaxel in the plasma compartment, with relatively stable levels being observed over the entire sampling-time period. As shown in Figure 2, the CSF to plasma concentration ratio of docetaxel varied in time with values for the total drug ratio increasing from 0.01 to 0.6% and the unbound drug ratio increasing from 0.1 to 9%.

Discussion

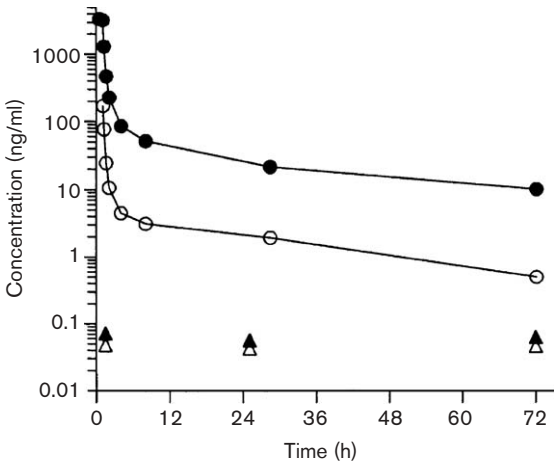
Despite numerous studies describing the clinical pharmacokinetics and pharmacodynamics of docetaxel

Table 1 Summary of docetaxel pharmacokinetics

Parameter	Patient 1[a]		Patient 2[b]	
	Total	Unbound	Total	Unbound
Plasma				
C_{max} (μg/ml)	1.18	0.119	3.21	0.169
AUC (μg·h/ml)	1.34	0.112	3.47	0.201
CL (l/h)	89.6	1067	36.0	620
$T_{1/2,z}$ (h)	12.4	13.3	33.9	23.6
CSF				
$C_{1.35\,h}$ (pg/ml)	NQ			
$C_{2.62\,h}$ (pg/ml)	NQ			
$C_{1.53\,h}$ (pg/ml)			71.6	47.7
$C_{25.1\,h}$ (pg/ml)			56.5	42.9
$C_{72.0\,h}$ (pg/ml)			63.1	46.5

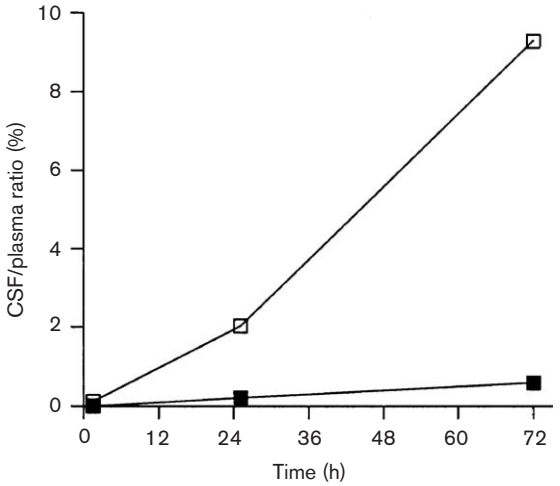
[a]Plasma samples taken up to 26 h.
[b]Plasma samples taken up to 72 h.
C_{max}, peak concentration; AUC, area under the plasma concentration–time curve extrapolated to infinity; CL, total clearance; $T_{1/2,z}$ (h), half-life of the terminal disposition phase; $C_{i\,h}$, concentration of docetaxel at *i* h after the start of infusion; NQ, not quantifiable (i.e. total CSF concentration below 40 pg/ml).

Fig. 1



Plasma concentration time curves of total (closed circles) and unbound (open circles) docetaxel in Patient 2. The triangles indicate the observed total (closed triangles) and unbound (open triangles) docetaxel concentrations in CSF samples.

Fig. 2



CSF to plasma concentration ratios of total docetaxel (closed symbols) and unbound docetaxel (open symbols) in Patient 2.

(reviewed in [3]), CSF pharmacokinetics and penetration for this agent have only been described previously in a single case report [6]. This current investigation adds to that knowledge because it is the first to take into account the vascular binding of docetaxel by measuring unbound concentrations. In addition, it reports on the application of a recently developed, highly sensitive assay based on liquid chromatography coupled with tandem mass spectrometric detection [1].

In both patients with metastatic breast cancer and leptomeningeal carcinomatosis studied here, only very low concentrations of docetaxel were measured in CSF, despite plasma levels of total docetaxel being within the therapeutic range associated with this regimen [2]. Interestingly, the docetaxel concentrations in CSF remained relatively constant over time, suggesting a very slow clearance from the CSF compartment relative to that in the systemic circulation. As a result, apparent equilibrium for docetaxel could not be determined within the time frame in which CSF samples were drawn. The limited surface area for docetaxel diffusion, the hydrophobic nature of the drug and transporter activity in the blood–brain barrier, combined with extensive vascular binding to serum proteins like α_1-acid glycoprotein [10] likely contributed to the slow equilibrium kinetics. For this reason, CSF represented only a relatively small additional compartment for docetaxel distribution, particularly in view of the large volume of distribution of docetaxel. It is of particular note that, because the CSF to plasma unbound concentration ratios are time dependent, single-point data are clearly inappropriate to directly assess the extent of CSF penetration by docetaxel. Furthermore, analysis based on total drug levels in plasma as done previously [6] potentially results in an estimated 10- to 20-fold underestimation of the extent of drug penetration in CSF.

Although the concentrations of docetaxel measured in CSF are relatively low, results of *in vitro* tests with several cell lines continuously exposed to docetaxel for 96 h previously suggested more than 50% inhibition of cell growth (IC_{50}) at a mean concentration of 0.4 ng/ml (5.1×10^{-10} M) [7]. Assuming a protein-bound fraction of approximately 90% for docetaxel in cell culture due to the presence of binding proteins in fetal calf serum, the IC_{50} for unbound docetaxel is around 40 pg/ml, which is comparable to values observed in the patient's CSF. Although the current data are limited to only two patients, the results cannot rule out that docetaxel administered i.v. at doses commonly used in 3-weekly treatment regimens (i.e. ≥ 75 mg/m^2), in some patients, might produces unbound drug levels in CSF for prolonged time periods that are associated with significant antitumor activity in experimental models and thus might have clinical relevance.

Acknowledgments

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