Topotecan Lacks Third Space Sequestration

Hans Gelderblom, Walter J. Loos, Jaap Verweij, Maja J. A. de Jonge, and Alex Sparreboom[1]

Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, 3075 EA Rotterdam, the Netherlands

ABSTRACT

The objective of this study was to determine the influence of pleural and ascitic fluid on the pharmacokinetics of the antitumor camptothecin derivative topotecan. Four patients with histological proof of malignant solid tumor received topotecan (0.45 or 1.5 mg/m^2) p.o. on several occasions in both the presence and absence of third space volumes. Serial plasma and pleural or ascitic fluid samples were collected during each dosing and analyzed by high-performance liquid chromatography for both the intact lactone form of topotecan and its ring-opened carboxylate form. The apparent topotecan clearance demonstrated substantial interpatient variability but remained unchanged within the same patient in the presence [110 ± 55.6 liters/h/m^2 (mean ± SD of eight courses)] or absence of pleural and ascitic fluid [118 ± 31.1 liters/h/m^2 (mean ± SD of seven courses)]. Similarly, terminal half-lives and area under the concentration-time curve ratios of lactone:total drug in plasma were similar between courses within each patient. Topotecan penetration into pleural and ascitic fluid demonstrated a mean lag time of 1.61 h (range, 1.37–1.86 h), and ratios with plasma concentration increased with time after dosing in all patients. The mean ratio of third space topotecan total drug area under the concentration-time curve to that in plasma was 0.55 (range, 0.26–0.87). These data indicate that topotecan can be safely administered to patients with pleural effusions or ascites and that there is substantial penetration of topotecan into these third spaces, which may prove beneficial for local antitumor effects.

INTRODUCTION

The increased risk of toxicity after chemotherapy in patients with pleural effusions and massive ascites is widely known and has been well documented for several compounds including methotrexate (1, 2) and fludarabine (3). This phenomenon is most likely related to greater drug accumulation in the peripheral compartment and a slower transport back to the central compartment, ultimately resulting in prolonged drug exposure. For this reason, it is advisable to evacuate large pleural and ascitic effusions before administration of these agents. On the other hand, penetration of the delivered chemotherapeutic agent should be sufficient to produce adequate drug distribution into the pleural or ascitic fluid to induce relevant local antitumor effects (4).

Diffusion of p.o. or systemically administered drugs into the peritoneum may be diminished by fibrous tissue due to prior surgery or prior regional i.p. chemotherapy, as reported for mitomycin C (4). In addition, several other factors including molecular weight, hydrophobicity, blood and lymph flow, and the capacity of the capillary wall and intervening interstitium have been shown to affect the peritoneal-blood barrier (5). The same factors may also be applicable for pleural effusions and the pleural fluid-blood barrier, although few paired plasma/pleural fluid pharmacokinetic data are available for antineoplastic agents (5–7).

In the absence of any pharmacokinetic data on third space sequestration for topotecan, a topoisomerase I inhibitor with substantial antitumor activity against various malignancies (reviewed in Ref. 8), we have prospectively evaluated the extent of penetration of this drug in pleural and ascitic fluid in cancer patients and assessed the influence of these third spaces on topotecan plasma pharmacokinetics.

MATERIALS AND METHODS

Patients and Treatment. Four patients with a histologically confirmed diagnosis of a malignant solid tumor that was metastatic and progressive after prior therapy were studied (Table 1). All patients had adequate hematopoietic (absolute neutrophil count $\geq 1.5 \times 10^9$/liter and platelet count $\geq 100 \times 10^9$/liter), hepatic (total serum bilirubin $< 1.25 \times$ upper normal limits), and renal (creatinine clearance ≥ 60 ml/min) function (9). The study drug topotecan was supplied as capsules containing either 0.25 or 1.0 mg of the active compound (SmithKline Beecham Pharmaceuticals Inc., Harlow, United Kingdom) and administered p.o. once daily, after an overnight fast, either for 5 consecutive days and repeated every 3 weeks (three patients) or for 2 consecutive days and repeated every week (one patient). In all four patients, comedication was uniform and consisted of cisplatin (50 or 70 mg/m^2 administered as a 3-h i.v. infusion immediately before topotecan on day 1 of every course) and ondansetron (8 mg, i.v.) combined with dexamethasone (10 mg, i.v.) given 30 min before cisplatin. During therapy, the patients did not use any other medication that might have interfered with topotecan absorption and disposition. The clinical protocol was approved by the institutional review board, and patients signed informed consent forms before entering the study.

Sample Collection. Material for pharmacokinetic analysis was collected during the first treatment course on days 1, 2, and 5 from patients on the 5-day schedule and during courses 1, 2, and 3 on days 1 and 2 from the patient on the 2-day schedule.

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[1] To whom requests for reprints should be addressed, at Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, Groene Hilledijk 301, 3075 EA Rotterdam, the Netherlands. Phone: 31-10-4391112; Fax: 31-10-4391053; E-mail: sparreboom@onch.azr.nl.

Table 1 Patient characteristics

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4
Age (yr)	41	65	40	35
Gender	M	M	M	F
Carcinoma	ACUP[a]	Rectum	ACUP	Ovarian
Third space	Pleural	Pleural	Ascites	Ascites
Treatment schedule	d1-5 q3w	d1-5 q3w	d1-5 q3w	d1-2 q1w
Drug dose (mg/m^2/day)	1.50	1.50	1.50	0.45
Drug dose (mg/day)	3.00	2.75	3.25	0.75 (d1) 1.00 (d2)[b]

[a] ACUP, adenocarcinoma of unknown primary origin; d1-5 q3w, once daily for 5 consecutive days, repeated every 3 weeks; d1-2 q1w, once daily for 2 consecutive days, repeated every week.

[b] As a result of body surface area-based dosing, and given the availability of 0.25-mg and 1.0-mg topotecan capsules only, the calculated weekly dose was split into unequal daily doses.

Blood samples were collected in 4.5-ml glass tubes containing lithium heparin as an anticoagulant (Becton Dickinson, Meylan, France) and were obtained before dosing and 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h after topotecan administration. The blood samples were placed immediately in an ice bath and centrifuged within 10 min at 3000 $\times$ *g* for 5 min at 4°C to separate the plasma. Subsequently, a volume of 250 μl of the plasma sample was added to 750 μl of ice-cold (−20°C) methanol in 2.0-ml polypropylene vials (Eppendorf, Hamburg, Germany). After vortex mixing for 10 s, the samples were stored at −80°C until the day of analysis. Pleural and ascitic samples were obtained at the same time points as blood samples using a Medicut 16GA cannula (45 $\times$ 1.7 mm, internal diameter; Sherwood Medical, Tullamore, Ireland) and collected in 4.5-ml polypropylene tubes after discarding the first 10 ml of fluid. These samples were processed as described above for plasma. To assess the extent of drainage of all fluid, ultrasonography or chest X-ray was performed after drainage.

Topotecan Assay. The samples, plasma as well as pleural liquid and ascites, were analyzed using reverse-phase high-performance liquid chromatography with fluorescence detection as described previously (10), with minor modifications. In brief, samples were centrifuged for 5 min at 23,000 $\times$ *g* at 4°C, followed by a 5-fold dilution in PBS before the injection of 200-μl aliquots into the high-performance liquid chromatography system. Chromatographic separations of topotecan carboxylate and lactone forms and endogenous compounds were achieved on a Hypersil BDS column (100 $\times$ 3 mm, internal diameter; 3-μm particle size; Shandon, Cheshire, United Kingdom) that was maintained at 35°C. The mobile phase, composed of 10 mM potassium dihydrogenphosphate-methanol-triethylamine (1750:500:4, v/v/v) with the pH adjusted to 6.0 (orthophosphoric acid), was delivered at a flow rate of 0.70 ml/min. The excitation and emission wavelengths of the Jasco FP920 fluorescence detector (Tokyo, Japan) were set at 381 and 525 nm, respectively, with an emission bandwidth of 40 nm. Chromatographic data analysis was performed based on peak height measurements relative to injected standards using the ChromCard system of Fisons (Milan, Italy).

Pharmacokinetic Analysis. Individual plasma concentrations of topotecan lactone and carboxylate forms were fit to a linear two-exponential equation using the Siphar version 4 software package (SIMED, Creteil, France), based on a variety of considerations including Akaike's and Schwarz' information criterion. The concentration-time profiles were obtained after zero-order input, with a weighted least-squares algorithm applying a weighting factor of $1/y$. The AUC[2] values were determined for both the lactone (AUC_L) and carboxylate forms (AUC_C) on the basis of the best fitted curves. The apparent plasma CL/f was calculated by dividing the dose administered by the observed AUC. The apparent terminal disposition half-life ($T_{1/2}$) was calculated as $\ln 2/k_{el}$, where k_{el} is the observed elimination rate constant of the terminal phase. The peak plasma concentrations (C_{max}) were determined graphically from the observed experimental values. The L:T ratio was defined as $AUC_L/(AUC_L + AUC_C)$. The fraction of drug penetrating into pleural or ascitic fluid was derived from the ratio of the topotecan total drug AUCs in the third space and plasma.

RESULTS

Plasma Pharmacokinetics. Peak plasma concentrations and AUCs of topotecan lactone after administration of a p.o. dose of 1.50 mg/m^2 to patients 1 and 2 were similar before and after pleural fluid was drained (fluid volumes, 3.1 liters and 1.1 liter, respectively; Table 2). Complete drainage of all fluid was confirmed by ultrasonography or chest X-ray after drainage. Data from patient 3, who had recurrent ascites during all topotecan administrations with volumes of 8.4 and 9.4 liters drained on days 2 and 6, respectively, indicated no difference in pharmacokinetic parameters between treatment days. Similarly, ascites (estimated to be 4.0 liters, 1.0 liter, and 1.0 liter on three occasions by ultrasonography and percutaneous drainage) had no measurable effect on topotecan plasma pharmacokinetics in patient 4 (Table 2). Overall, the CL/f demonstrated substantial interpatient variability but remained unchanged within the same patient in the presence [110 $\pm$ 55.6 liters/h/m^2 (mean $\pm$ SD; eight courses)] or absence of pleural or ascitic fluid [118 $\pm$ 31.1 liters/h/m^2 (mean $\pm$ SD; seven courses)]. Topotecan L:T ratios in plasma were very similar between courses within each patient and averaged 40.0 $\pm$ 3.89% (drained) and 40.0 $\pm$ 6.11% (not drained), respectively.

Pleural and Ascitic Fluid Penetration. Given the low plasma protein binding of topotecan (~35%; Ref. 11) and the relatively high total protein content in pleural fluid and ascites of the patients (range, 38–45 mg/ml), no correction for protein binding was performed. Topotecan concentrations in pleural fluid and ascites peaked at ≥6 h after oral dosing, demonstrating a mean lag time of 1.61 h (range, 1.37–1.86 h; overall mean $\pm$ SD in plasma, 0.63 $\pm$ 0.28 h), and rose slowly to equal that in plasma by ~8 h (Fig. 1, *A–C*). Topotecan disappearance from pleural fluid [apparent $T_{1/2}$, 12.0 h ($n = 1$)] and ascites [apparent $T_{1/2}$, 8.0 h ($n = 1$)] appeared to be slower than that from plasma. As a result, third space penetration, expressed as the ratio of concomitant pleural fluid or ascites:plasma concentration of total topotecan, depended greatly on the sampling time point and

[2] The abbreviations used are: AUC, area under the concentration-time curve; CL/f, topotecan clearance; L:T, the ratio of the systemic exposure of topotecan lactone to total drug.

Table 2 Summary of topotecan plasma pharmacokinetics in the presence or absence of pleural or ascitic fluid[a]

Patient no.	Third space	No. of curves	AUC_L[b] (ng·h/ml)	CL/f (liters/h/m^2)	C_{max} (ng/ml)	$T_{1/2}$ (h)	L:T ratio (%)
1	Pleural	1	12.9	136	2.06	1.83	44.7
	None	2	14.7, 18.3	119, 95.5	3.33, 6.50	2.17, 1.77	44.8, 43.5
2	Pleural	1	23.3	64.3	2.71	4.53	36.3
	None	2	18.7, 17.0	80.0, 88.1	2.04, 2.30	4.43, 5.90	34.6, 35.8
3	Ascites	3	22.1 ± 1.92	68.2 ± 5.82	2.43 ± 0.32	4.38 ± 0.36	40.3 ± 2.42
4	Ascites	3	5.80 ± 1.82	155 ± 61.2	1.51 ± 0.65	3.00 ± 0.58	39.2 ± 5.3
	None	3	6.28 ± 1.42	148 ± 12.1	1.16 ± 0.15	3.40 ± 0.66	40.5 ± 8.4

[a] Data were obtained in both the presence and absence of third space fluids in each individual patient treated with topotecan doses, and the treatment schedules were as given in Table 1. The AUC values were calculated by compartmental analysis, and data of patients 3 and 4 represent mean values ± SD.

[b] AUC_L, area under the topotecan lactone plasma concentration-time curve; C_{max}, peak plasma concentration of topotecan lactone; $T_{1/2}$, apparent terminal disposition half-life.

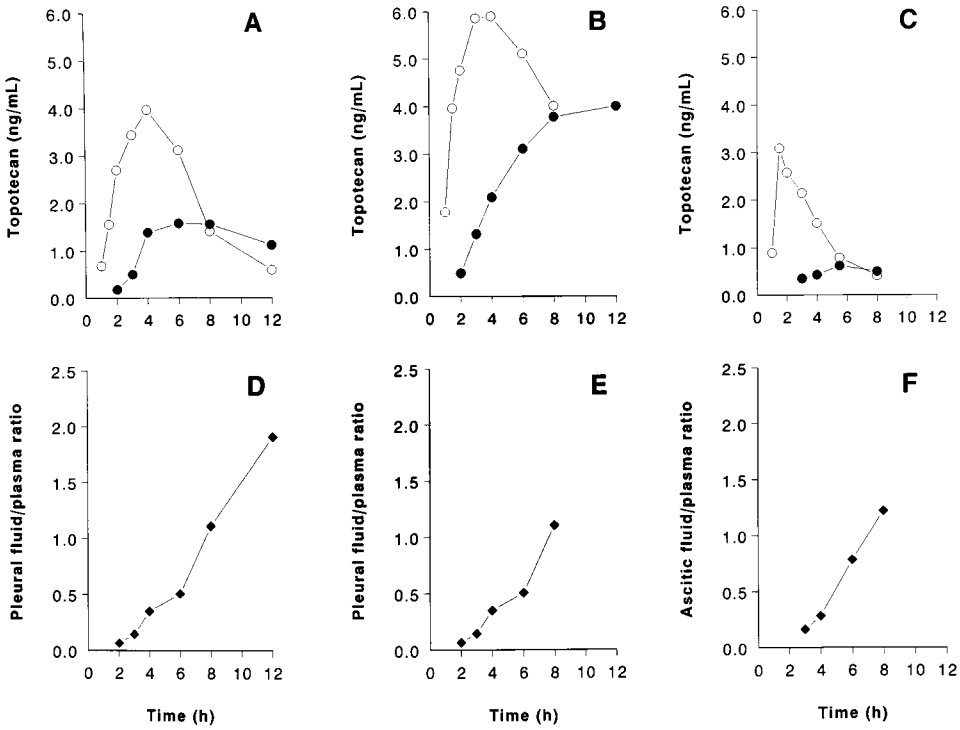

Fig. 1 Concentration *versus* time plots of topotecan expressed as total drug (lactone plus carboxylate) in plasma (○) and ascites (patients 1 and 2) or pleural fluid (patient 4; ●) and corresponding topotecan third-space fluid:plasma concentration ratios (◆) in patients 1 (*A* and *D*), 2 (*B* and *E*), and 4 (*C* and *F*).

increased significantly with time in all patients (Fig. 1, *D–F*). Overall, the mean ratio of third space topotecan total drug AUC to that in plasma was 0.55 (range, 0.26–0.87). The hydrolysis of topotecan to the ring-opened form was rapid, and L:T AUC ratios were 18.1% and 23.5% in pleural fluid and 29.2% in ascites. Measurement of topotecan in ascites from patient 3 indicated that less than 1% of the administered dose was present in ascites at 6–8 h after dosing, indicating the lack of a sink effect.

Toxicity. Overall, treatment was very well tolerated in the four patients. No severe hematological toxicity or other organ toxicity was observed after p.o. topotecan administration at these doses. The third patient experienced fatigue (grade 2 on a 4-point scale, National Cancer Institute Common Toxicity Criteria), whereas the fourth patient had mild nausea and vomiting.

DISCUSSION

This study was performed to explore the influence of pleural and ascitic fluid on the pharmacokinetic behavior of topotecan in cancer patients. Although the topotecan administration was preceded by cisplatin infusion in this study in all patients, important pharmacokinetic interactions that may have influenced the generated data are not very likely: (*a*) comparison of the kinetics of topotecan in clinical combination therapy regimens with cisplatin with the kinetics of single-agent therapy did not reveal an apparent interaction (12); and (*b*) using a randomized cross-over design for the administration order, no statistically significant differences in clinical pharmacokinetics were observed between sequences of p.o. topotecan and i.v. cisplatin (9).

Topotecan concentrations in pleural fluid and ascites were

initially less than those in plasma, and several hours were required for equilibrium to be attained between these fluids and plasma. The limited surface area for topotecan diffusion relative to the volumes of fluid and the fact that pleural fluid and ascites are not well stirred, in addition to the hydrophilic nature of the drug, likely contributed to the slow equilibrium kinetics. Overall, both pleural fluid and ascites represented only a small additional compartment for topotecan distribution, particularly in view of the already large topotecan steady-state volume of distribution of 73-133 liters (13). Nevertheless, concentrations equivalent to that in plasma were achieved after 8 h, and topotecan appeared to be more slowly eliminated from the pleural and peritoneal cavity than from plasma. This is in keeping with earlier findings indicating slow peritoneal clearance of topotecan and high peritoneal:plasma concentration ratios of >10 after i.p. drug administration (14, 15).

Topotecan has been detected previously in ascites of two patients treated with a combination of i.v. topotecan and p.o. etoposide (16). However, the reported ascitic fluid:plasma concentration ratios were established by single-point measurements at different times after administration. Because these concentration ratios were shown in our patients to be by no means constant parameters during the dosing interval (Fig. 1, *D–F*), single-point data are clearly inappropriate to directly compare the extent of penetration by topotecan. Hence, the approach of using paired AUC values in third space fluids and plasma, as performed in the present study, should be considered the gold standard to report these ratios. Although the described data on topotecan accumulation are limited to only four patients, our results suggest that oral administration of topotecan can produce adequate drug distribution in pleural fluid and ascites at concentrations associated with significant antitumor activity in experimental models (17, 18). In this context, it is of particular interest that topoisomerase I inhibitors were previously shown to be highly S-phase specific and that cytotoxicity is a function of the time to drug exposure above a certain threshold concentration (19). The topotecan penetration and subsequent accumulation in the third spaces thus might offer a potential therapeutic advantage in that tumor cells in the thoracic and peritoneal cavity are exposed to high local drug levels for prolonged time periods. This concept has also been described recently for systemic therapy with the structurally related camptothecin derivative irinotecan, although in contrast to topotecan, concentrations appeared to decline in parallel with those in plasma (20). The reason for this discrepant behavior is unknown, but it is likely to reflect intrinsic differences in physicochemical and/or pharmacokinetic properties of both compounds, including differential binding to (plasma) proteins.

The plasma pharmacokinetics of topotecan revealed a substantial degree of interindividual variability, in line with previous observations (9).[3] By comparing topotecan plasma levels in the same patient before and after drainage of pleural or ascitic fluid, no differences in rate of absorption and elimination became apparent. The lack of increased systemic exposure to topotecan in patients with massive third space volumes was further substantiated by the lack of excess toxicity. Hence, in contrast to clinical information on irinotecan treatment that suggested an increased risk of severe toxicity in patients with large pleural effusions or ascites (20), there was no evidence that the severity of toxicity differed between study courses with and without third space volumes in our patients treated with topotecan.

In conclusion, we have shown that: (*a*) topotecan plasma pharmacokinetics are unaltered in patients with third space volumes; (*b*) topotecan can be safely administered to patients with large pleural effusions or massive ascites; and (*c*) there appears to be a substantial penetration of topotecan into these third spaces, which may prove beneficial for local antitumor effects.

[3] W. J. Loos, H. Gelderblom, J. Verweij, M. J. A. de Jonge, and A. Sparreboom. Inter- and intrapatient variability in oral topotecan pharmacokinetics: implications for body surface area dosage regimens, submitted for publication.

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