Red blood cells: a neglected compartment in topotecan pharmacokinetic analysis

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Previously, a gender dependency of topotecan was found in the pharmacokinetics in the plasma compartment. Here, we prospectively studied the red blood cell (RBC) partitioning of topotecan and evaluated its consequences for overall drug disposition. Blood samples were obtained from 12 patients receiving cisplatin followed by i.v. topotecan. Topotecan pharmacokinetic analysis was performed in whole blood, plasma and RBCs. Significantly slower clearance was noted in females ($n=7$) compared to males ($n=5$) for lactone and total topotecan in plasma ($p<0.0001$), and for total drug in RBCs ($p=0.027$), but not in whole blood. In addition, no gender-dependent differences were observed in the terminal half-lives of topotecan in any of the compartments. The area under the curve ratios for RBC total to plasma lactone were 2.53 ± 0.0640 and 2.13 ± 0.442 in males and females, respectively. Hence, topotecan displays preferential affinity for RBCs compared to plasma, although these cells do not act as a depot in which drug accumulates over time. RBCs thus play a principal role in the distribution kinetics of topotecan and have a major impact on its plasma pharmacokinetics. The data warrant a change from current practice in pharmacokinetic studies with this agent and provide further evidence that, in general, the choice of the appropriate assay matrix should be rationally based. *Anti-Cancer Drugs* **14:227–232**

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Introduction

Topotecan (Hycamtin) is a water-soluble, semisynthetic analog of the topoisomerase I inhibitor camptothecin with a complex pharmacokinetic profile that originates mainly from a spontaneous pH-dependent interconversion between a lactone and carboxylate form of the drug [1] (Fig. 1). The latter form not only lacks the ability to passively diffuse across cell membranes, unlike the intact lactone, but also does not stabilize the cleavable complex between DNA and the nuclear enzyme topoisomerase I, and, consequently, lacks cytotoxic activity [2]. During the last decade, the pharmacokinetics of topotecan have been extensively examined with the drug administered either as an i.v. infusion [3–6] or when given orally [7–9]. Furthermore, relationships have been documented between the systemic exposure to topotecan lactone or total topotecan (i.e. lactone plus carboxylate forms) as measured in plasma and the dose-limiting toxicity, neutropenia. Topotecan has a plasma protein binding of approximately 35% and hence 65% of the drug is in principle directly available for cellular uptake. Since only the non-dissociated topotecan lactone form can passively cross cell membranes, including those of red blood cells (RBCs), the concentration of topotecan might differ importantly between whole blood, RBCs and plasma. Unfortunately, it is currently routine practice to measure drug concentrations in plasma or serum and only occasionally in whole blood or RBCs [10–15]. Apparently, the choice of the appropriate matrix is not always rationally based and factors such as magnitude of RBC partitioning are rarely considered in the decision-making process. Based on theoretical considerations, it has been argued that whole blood, as the physiologically meaningful body fluid, rather than plasma is the more appropriate reference matrix for calculating and interpreting clearances and volumes of distribution [16]. The study described here was designed to prospectively evaluate the RBC partitioning of topotecan in male and female patients using a recently developed high-performance liquid chromatographic (HPLC) method [17], and evaluate the consequences for overall drug disposition profiles.

Materials and methods

Eligibility

Patients with a histologically or cytologically confirmed diagnosis of a malignant solid tumor, refractory to standard therapy or for which no recognized therapy was available, were eligible for this study. Additional eligibility criteria included: age between 18 and 75 years;

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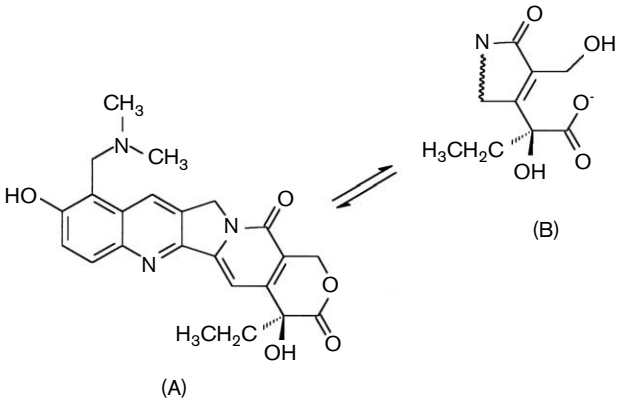
Fig. 1



Chemical structures of the lactone (A) and carboxylate (B) forms of topotecan.

Eastern Cooperative Oncology Group performance status ≤ 1; adequate hematopoietic [white blood cells (WBC) $\geq 4.0 \times 10^9$/l, absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/l and platelet count $\geq 100 \times 10^9$/l], hepatic (bilirubin within normal limits, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 2 times the upper limit of institutional normal) and renal function (creatinine clearance ≥ 60 ml/min). All patients should have an estimated life expectancy ≥ 12 weeks and no previous chemotherapy was allowed for at least 4 weeks prior to enrollment. The study protocol was approved by the Erasmus MC Ethics Board and written informed consent before study entry was obtained from all patients.

Treatment and drug administration

Glaxo SmithKline (Harlow, UK) supplied the lyophilized vial preparations containing 5 mg of topotecan lactone. The drug was administered once daily for 5 consecutive days in 50 ml of 0.9% saline as a 30-min i.v. infusion at dose levels of 0.50 or 0.75 mg/m^2. On the first treatment day, during course 1 or 2, topotecan administration was preceded by a single 3-h i.v. infusion of cisplatin (Pharmachemie, Haarlem, The Netherlands) in 250 ml of 3% saline, at a dose level of 50 mg/m^2.

Sample collection and processing

Blood samples for topotecan pharmacokinetic analysis were collected in 4.5-ml glass tubes containing lithium heparin as anticoagulant. Sample collection was performed prior to dosing, at 15 min after the start of infusion, immediately before the end of infusion, and at 15 and 30 min, and 1, 2, 3, 4, 6, 8, and 10 h after the end of infusion. All samples were immediately placed on ice and processed as described below within 10 min after collection.

Drug measurement

A detailed description of the procedure for separation of plasma and unwashed RBC has been reported elsewhere [17]. In brief, aliquots of 1.5 ml of whole blood were centrifuged in MESED instruments (Fabre, Kelmis, Belgium) for 10 min at 3600 g. After the first centrifugation step, 250 μl of plasma supernatant was transferred into a 2 ml polypropylene tube containing 750 μl of ice-cold methanol, which stabilizes the lactone to carboxylate ratio. Subsequently, the MESED instrument was centrifuged again for 10 min at 3600 g, leaving an aliquot of 102 μl of packed unwashed RBCs in the instrument, which were harvested by a third centrifugation step of 5 min at 200 g. The deproteinized plasma sample, the RBC fractions, as well as whole blood were stored at $T < -70$°C until analysis. Simultaneous determination of the lactone and carboxylate forms of topotecan was performed by HPLC with fluorescence detection [18] with minor modifications [9], while concentrations of topotecan in whole blood and RBCs were determined by a recently described method [17].

Pharmacological analysis

Pharmacokinetic profiles were analyzed using the software package Siphar version 4.0 (InnaPhase, Philadelphia, PA) by two- or three-compartment models after zero-order input. The AUC values were extrapolated to infinity as calculated from the model based on the best-fitted curves. The clearance values were calculated by dividing the dose in mg/m^2 or the absolute dose in mg by the observed AUC, and were expressed in l/h/m^2 and l/h, respectively. The terminal disposition half-lives were calculated as ln2/k, where k is the rate constant of the terminal disposition phases in the different matrices (expressed in h^{-1}). The AUC ratio of topotecan lactone to total drug in plasma and the AUC ratio of total topotecan in RBCs to the lactone form in plasma, based on the individual concentrations in each patient, were also calculated.

Statistical considerations

All parameters are reported as mean values ± SD. Two-tailed unpaired Student's t-tests were performed to evaluate statistically significant differences in pharmacokinetic and biochemical parameters between males and females, using the NCSS package (version 5.X; J.L. Hintze, Kaysville, UT). One-way analysis of variance (ANOVA) was used to evaluate statistical differences between groups, using the same program. Linear regression analysis was performed using the same program to evaluate relationships between different parameters of interest. Probability values of < 0.05 were regarded as significantly different in all tests applied.

Results

Patient characteristics

Twelve patients received the combination treatment in which topotecan was preceded by cisplatin administered

Table 1 Summary of patient characteristics

	Males	Females	*p*
N	5	7	
Age [years (median, range)]	54, 36–60	46, 39–68	
Dose [(mg/m^2) 0.50/0.75]	1/4	0/7	
BSA (m^2)	1.85 ± 0.0563	1.70 ± 0.177	0.10
Hematocrit (l/l)[a]	0.35 ± 0.035	0.33 ± 0.035	0.39
Creatinine (μmol/l)	74.0 ± 7.48	64.0 ± 16.1	0.23
Bilirubin (μmol/l)	5.2 ± 3.4	5.4 ± 1.9	0.90
ALT (U/l)	21.4 ± 10.8	17.4 ± 9.59	0.51
AST (U/l)	25.4 ± 6.99	23.3 ± 6.71	0.61
Albumin (g/l)	39.2 ± 4.15	39.3 ± 2.21	0.96
Total protein (g/l)	71.0 ± 16.9	73.0 ± 3.37	0.76

[a]$n = 4$ for males, due to missing data.
Probability values unpaired in Student's *t*-test.

in 250 ml of 3% saline and were all evaluable for pharmacokinetics. No significant differences were observed in any of the characteristics between male and female patients (Table 1).

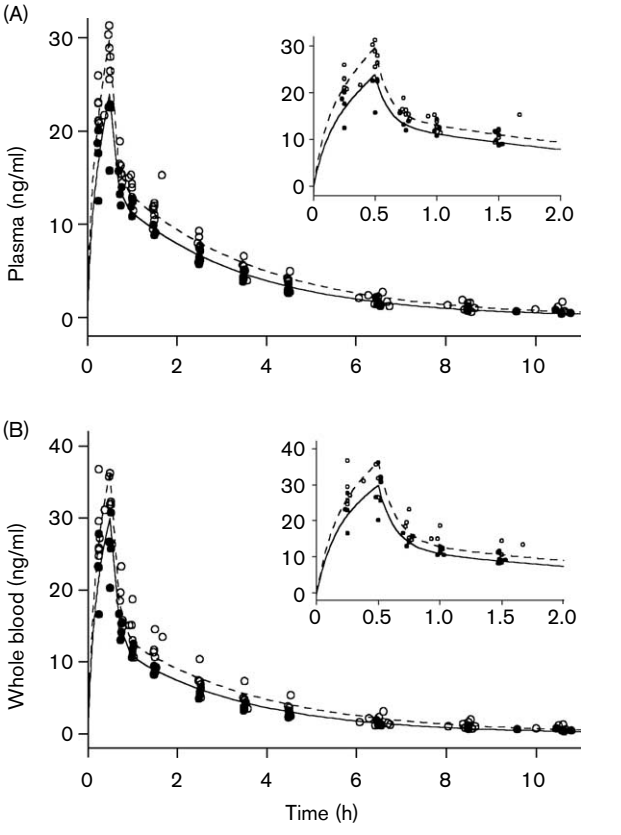
Topotecan blood distribution

The individual data points of the concentrations of total topotecan in plasma and whole blood after topotecan administration at a dose of 0.75 mg/m^2 to seven females and four males (only one patient received 0.50 mg/m^2) are shown in Fig. 2, with the curves simultaneously fitted to a two-compartment model. Significant gender-dependent differences were noted in the plasma clearance of topotecan lactone and total drug as well as in RBC clearance, when expressed in l/h (Table 2). After correction for individual body surface area (BSA) values of all patients, the plasma clearance remained significantly different between males and females, while differences in RBC clearance of total topotecan disappeared. In contrast, no significant differences were noted in whole blood clearance of topotecan, either when expressed in l/h or in l/h/m^2. These effects on clearance were not accompanied by gender-related differences in the half-lives of the terminal disposition phase of topotecan in the various matrices. In addition, these values were identical between the matrices ($p = 0.11$; one-way ANOVA). The concentration ratio of total topotecan in RBCs to topotecan lactone in plasma was found to be constant during the entire concentration–time curve and independent of lactone concentrations (Fig. 3A; $R = 0.999$, $p < 0.0001$). This concentration ratio of total topotecan RBCs to topotecan lactone in plasma was linearly correlated with hematocrit values of the individual patients (Fig. 3B; $R = 0.53$, $p = 0.065$). In Figure 4, the significant linear relationship ($R = 0.78$, $p = 0.0018$) between the AUC ratios of plasma lactone to total and the RBCs total to plasma lactone in the individual patients is shown.

Hematological pharmacodynamics

The nadir values in males and females of WBC (1.27 ± 0.93 versus 1.45 ± 0.74 × 10^9/l), ANC (0.38 ± 0.55 versus 0.38 ± 0.31 × 10^9/l) and platelets (60 ± 27 versus 85 ± 77 × 10^9/l) were not significantly different.

Fig. 2



Simultaneous two-compartmental fit of the total topotecan concentrations in plasma (A) and whole blood (B) in seven females (open symbols and dotted line) and four males (closed symbols and solid line) after administration of 0.75 mg/m^2 topotecan. The inserts show the kinetic profiles during the first 2 h after the administration of topotecan.

Discussion

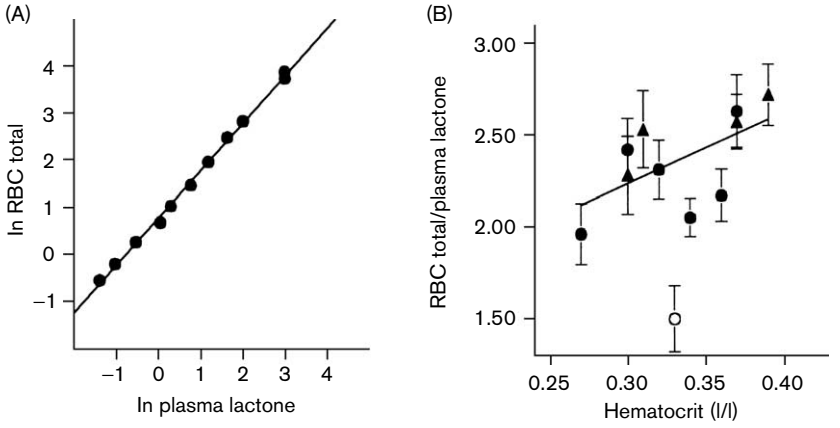
The cellular compartments of blood are commonly neglected in pharmacokinetic analysis of (anticancer)

Table 2 **Summary of the topotecan pharmacokinetics**

	Males ($n=5$)	Females ($n=7$)	p
Clearance (l/h)			
plasma lactone	83.2 ± 8.95	54.1 ± 4.54	<0.0001
plasma total	29.6 ± 1.40	21.6 ± 2.11	<0.0001
RBC total	32.9 ± 3.67	26.2 ± 4.88	0.027
whole blood total	28.3 ± 3.75	22.4 ± 5.37	0.061
Clearance (l/h/m^2)			
plasma lactone	44.8 ± 6.26	32.0 ± 2.33	0.0005
plasma total	16.1 ± 1.62	12.9 ± 1.26	0.0031
RBC total	17.7 ± 2.47	15.8 ± 3.28	0.30
whole blood total	15.2 ± 2.07	13.5 ± 3.40	0.35
Terminal half-live (h)			
plasma lactone	2.63 ± 0.642	3.65 ± 1.16	0.12
plasma total	2.59 ± 0.900	2.27 ± 0.251	0.42
RBC total	2.25 ± 0.254	2.69 ± 0.776	0.25
whole blood total	2.58 ± 0.464	2.95 ± 0.986	0.50
AUC ratios			
plasma lactone/plasma total	0.362 ± 0.0138	0.400 ± 0.0276	0.019
RBC total/plasma lactone	2.53 ± 0.0640	2.13 ± 0.442	0.074

Probability values unpaired in Student's *t*-test.

Fig. 3

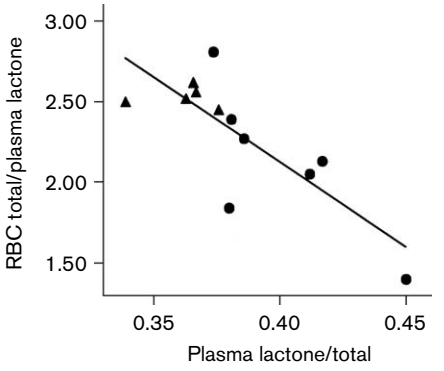


(A) Representative linear relationship between topotecan lactone concentrations in plasma and total topotecan concentrations in RBCs of a single patient, and (B) relationship between hematocrit and the concentration ratio of total topotecan in RBCs to topotecan lactone in plasma. Circles, data from female patients; triangles, data from male patients; the open circle was not used for the calculation of the relationship. The ratios are presented as mean values ± SD.

drugs. However, agents might bind to or accumulate in various circulating cells, as has been described for some Vinca alkaloids and paclitaxel with human platelets [19,20] or various other agents with RBCs [10–15]. Obviously, clinically meaningful relationships between concentrations in the plasma fraction and drug effects (i.e. toxicity and efficacy) can only be made if the drug is equally distributed among the different compartments, when blood distribution is not affected by xenobiotic excipients (e.g. delivery vehicles [21]), and when this process is time and concentration independent [15]. Especially in the case when drugs are substantially bound to or accumulated in RBCs, these cells might play an important role in the pharmacokinetic behavior of the drugs, since the RBCs form a major compartment of blood [10].

Previously, a significant gender-dependent difference in the plasma pharmacokinetics of topotecan lactone after oral administration was noted, which we attributed to an intrinsic difference in hematocrit between males and females [22]. In the present study, in which topotecan was administered as a 30-min i.v. infusion, we similarly found a significantly higher plasma clearance of topotecan lactone (i.e. 54%), as well as a significant increase of 37% in total topotecan plasma clearance in males compared to females. These differences remained significant even after correction for the individual BSA values, with differences of 40 and 25% for the lactone and total form, respectively. In contrast, the significant difference of 26% in the absolute RBC clearance of total topotecan disappeared after correction for BSA, while in whole blood no differences were observed in the absolute and

Fig. 4



Linear relationship between the AUC ratios of plasma lactone to total and the RBC total to plasma lactone in the individual patients. Circles, data from female patients; triangles, data from male patients.

BSA corrected clearance of total topotecan. In addition, the terminal disposition half-lives were not different between males and females, while also no differences were noted between the different matrices. These observations clearly suggest that there is a difference in the topotecan blood distribution between males and females; indeed, the AUC ratio of topotecan lactone to total drug in plasma was significantly lower in males compared to females (i.e. 10%), which is also in line with data obtained after oral administration or continuous infusion of topotecan [22]. The AUC ratio of total topotecan in RBCs to topotecan lactone in plasma was not significantly different between males and females, although males showed on average a 19% higher ratio. The lack of significance is most likely due to the small group of patients studied and the highly variable hematocrit values among cancer patients. However, a correlation was observed between individual hematocrit values and the RBC to plasma lactone ratio, which provides further support for the hypothesis that hematocrit is one of the predictors of topotecan clearance in plasma [22], and not the gender itself, as described in a pharmacokinetic model [23]. The relatively high RBC to plasma ratios show that the lactone form of topotecan preferentially binds to RBCs and these cells serve as a major compartment for topotecan distribution in the systemic circulation. In addition, the RBC to plasma ratios were constant over time in the studied patients, independent of the lactone concentration or the sampling time point for blood collection. This indicates that RBCs do not act as a depot for topotecan in which the drug accumulates over time, as has been described for other drugs, including lometrexol [10,11]. In contrast, the ratio between the total topotecan concentration observed in plasma versus the RBC concentration of total drug decreased over time. Moreover, one of the studied female patients had a substantially lower RBC to plasma ratio (i.e. 1.40) as compared to the other patients. This low value was accompanied by a relatively high plasma lactone to total ratio of 0.450. The underlying reason for these discrepant ratios is as yet unknown, but might be related to altered binding of topotecan to plasma proteins resulting in stabilization of the lactone form in the plasma compartment. Alternatively, a change in RBC membrane permeability might also cause the altered ratios.

A strong linear correlation was noted between the two concentration ratios, with higher RBC distribution at low plasma lactone to total ratios, also suggesting that an equilibrium of topotecan lactone exists between plasma and RBC. In addition, this suggests that the lactone form of topotecan is in equilibrium with both the carboxylate form in plasma and the part distributed to the RBCs, as has been postulated earlier [10]. The observations presented here support the earlier presented findings that the lipophilic lactone form of camptothecins interacts with the lipid bilayer of RBCs and so stabilizes the lactone moiety [24]. In addition, in the case of the related agent, 9-aminocamptothecin, the pharmacokinetic profile in plasma after bolus i.v. infusion was characterized by the presence of a secondary peak occurring 2–3 h after the end of the infusion as a result of initial uptake of the lactone form by RBCs during the infusion, followed by accumulation of the carboxylate form in the plasma compartment [25].

Overall, these data indicate that RBCs play an important role in the blood distribution of topotecan, which might have implications for the proper evaluation of exposure–toxicity relationships, which are currently based solely on measurement of topotecan lactone or total drug in plasma and have been used as a basis for the development of a population model for topotecan pharmacokinetics in order to refine dosing strategies [23]. In addition, the data in this manuscript support the hypothesis that the gender-dependent differences in the plasma pharmacokinetics of topotecan are the result of the physiological difference in hematocrit between males and females [22].

The findings presented here may be of great importance for future individualized dosing strategies of topotecan, in order to achieve more efficient and less toxic therapies, which should not be based on the pharmacokinetics in the plasma compartment and the gender of the patient [23]. When the nature of the interpatient variability in drug outcome is better understood, for which additional studies and the development of pharmacokinetic/pharmacodynamic models are needed, which further investigate the role of the blood distribution of topotecan with respect to antitumor activity and/or toxicity,

individualized dosing of topotecan will be feasible. The observations presented here might not only be relevant for topotecan, but also for other camptothecin analogs. Currently, the blood distribution patterns of several structurally related analogs (including irinotecan and its metabolite, SN-38) are under investigation [26].

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