Anti-Cancer Drugs 2000, **11,** pp. 673–680

Clinical report

Gender-dependent pharmacokinetics of topotecan in adult patients

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Gender-dependent differences in the clinical pharmacokinetic behavior of various drugs have been documented previously. Most commonly, these differences are associated with differences in body composition, renal elimination, drug absorption or hepatic metabolism. Gender-dependent differences in the pharmacokinetics of topotecan (Hycamtin®) have not yet been described. In this report, pharmacokinetic data of the lactone and carboxylate forms of topotecan were derived from clinical studies in which topotecan was administered either orally or i.v. to a total of 55 males and 37 females. A significant difference (p=0.0082) of 38% was found between the apparent clearance of topotecan lactone after oral administration in males (237 ± 105 l/h) and females (163 ± 62.5 l/h). When adjusted for body surface area, this difference remained significant (p=0.031). Similarly, differences were noted in the percentage of topotecan in the lactone form (37.1 ± 5.32 versus 41.7 ± 6.51%, p=0.0076). Statistical analysis revealed that individual hematocrit values, which were consistently lower in females ($p < 0.023$), were a significant predictor of the apparent topotecan lactone clearance. This was confirmed experimentally in *in vitro* incubation studies in whole blood using artificially altered hematocrit values and in blood samples from both male and female volunteers. Topotecan is thus subject to significant gender-dependent differences in pharmacokinetics that arise as a result of a physiological difference in hematocrit values between males and females. This finding may have significant implications for the interpretation of the relationships between pharmacokinetics and pharmacodynamic outcome of topotecan treatment, and may provide a basis for the development and refinement of future clinical protocols. [© 2000 Lippincott Williams & Wilkins.]

Key words: Blood distribution, hematocrit, *in vitro*, lactone, topotecan.

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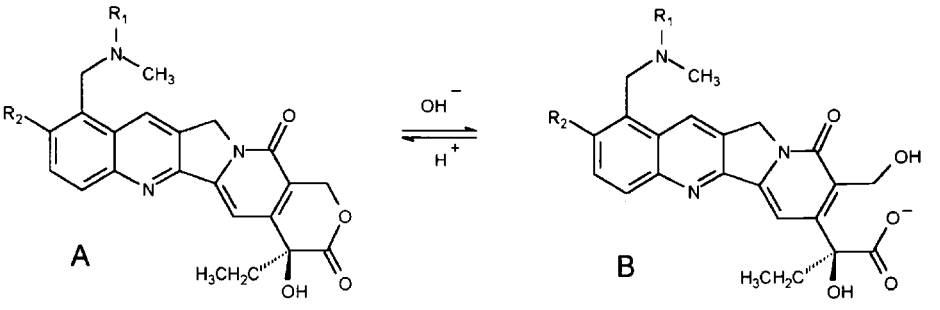
Introduction

Topotecan (Hycamtin®; Figure 1), a water-soluble semisynthetic analog of the topoisomerase I inhibitor camptothecin, is one of the most promising new anticancer agents. Single-agent topotecan, administered i.v., has demonstrated antitumor activity against various solid tumors in adult cancer patients, including metastatic ovarian and small cell lung cancer. Most responses were achieved using a daily $\times 5$ schedule in which topotecan was administered as a 30 min infusion.[1] Since daily i.v. administration of topotecan for 5 days, repeated every 21 days, is inconvenient for patients, an oral formulation of topotecan has been developed with a bioavailability of 42 ± 13%.[2] Different administration schedules of oral topotecan have been evaluated in clinical studies, including once daily $\times 5$ and 10 and twice daily $\times 10$ and 21 administrations, from which the once daily $\times 5$ schedule was recommended for future clinical studies.[3] The need for further clinical development of the oral topotecan formulation became even more important in view of recent findings that the oral formulation has similar efficacy as compared to the i.v. formulation, while less hematological toxicity was observed.[4,5]

The pharmacokinetic profile of topotecan is quite complex since it can undergo a spontaneous pH-dependent interconversion between a pharmacologic active lactone form and an inactive carboxylate form (Figure 1). Gender-dependent differences in pharmacokinetic behavior have been described for a wide variety of compounds over the last few decades. Most commonly, these have been shown to be associated with differences in body composition, renal elimination, drug absorption or hepatic function.[6] In this report, we describe gender-dependent differences in topotecan pharmacokinetics after both



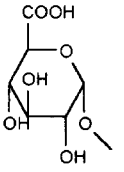
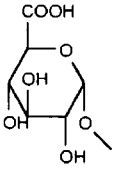
Compound	R1	R2
Topotecan	CH_3	OH
N-desmethyl topotecan	H	OH
Topotecan-*O*-glucuronide	CH_3	
N-desmethyl topotecan-*O*-glucuronide	H	

Figure 1. Chemical structures of the lactone (A) and carboxylate (B) forms of topotecan and its known human metabolites.

oral and i.v. administration, and present *in vitro* studies to provide a formal explanation of this phenomenon.

Patients and methods

Patient selection criteria

All patients included in the studies had a histologically or cytologically confirmed diagnosis of a malignant solid tumor, refractory to standard therapy or for which no recognized therapy was available. The patients participated in either a phase I study, in which oral topotecan was combined with i.v. cisplatin,[7] or a phase II study of single-agent topotecan administered as a 21-day continuous i.v. infusion.[8] The eligibility criteria, treatment plans and detailed clinical profiles have been fully described elsewhere.[7,8]

Drug administration

SmithKline Beecham Pharmaceuticals (Harlow, UK) supplied capsules containing either 0.25 or 1.0 mg of topotecan lactone and a lyophilized vial preparation containing 5 mg of topotecan lactone. Orally administered topotecan was studied at dose levels of 0.75,

1.00, 1.25, 1.50, 1.75, 2.00 or 2.30 mg/m^2/day for 5 days, repeated every 3 weeks, in combination with a fixed dose of 75 mg/m^2 cisplatin by a 3-h infusion, in 49 patients.[7] A total of 10 patients, treated with oral topotecan daily $\times 5$, at dose levels of 1.50 or 1.75 mg/m^2, preceded by i.v. cisplatin at a dose of 50 mg/m^2 on day 1 of each course, was also included in this study. In the i.v. phase II study, topotecan was administered as a 21-day continuous infusion at dose levels of 0.50 and 0.60 mg/m^2/day at an infusion rate of 6 ml/24 h, using ambulatory pumps, repeated every 28 days.[8]

Blood sample collection and analysis

Blood samples were collected in 4.5 ml glass tubes containing lithium heparin as anticoagulant. Following oral administration, samples were obtained prior to dosing, and 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after dosing on day 1.[7] Similarly, in the i.v. trial blood was withdrawn prior to infusion and at steady state on day 8 after start of dosing.[8] The blood samples were centrifuged immediately at the site of the patient to separate the plasma. The plasma samples were directly deproteinized by 4-fold dilution in ice-cold (-20°C) methanol, resulting in a stabilized lactone to carboxylate ratio[9] and stored at -80°C upon analysis. Simultaneous determination of the lactone and carboxylate form of topotecan was performed by a reversed-phase high-performance liquid chromatographic method as described.[9]

Pharmacokinetic analysis

In the present pharmacokinetic analysis we used the pharmacokinetic data of day 1 of course 1 in the oral phase I study[7] and of day 8 in the continuous infusion phase II study.[8] The area under the plasma concentration–time curve (AUC) of total topotecan, i.e. lactone plus carboxylate, and the topotecan lactone and carboxylate forms in the oral phase I study were calculated by non-compartmental and two- or three-compartmental analysis models after zero-order input. The apparent clearance of topotecan lactone and the clearance of total topotecan in the oral phase I study 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. While in the i.v. study, the clearances were calculated by dividing the rate of infusion divided by the steady-state plasma concentration. The apparent terminal disposition half-life of topotecan lactone and carboxylate were calculated as $\ln 2/k$, in which k represents the rate constant of the terminal disposition phase. The lactone to total ratio in the oral phase I study was defined as the AUC_L/AUC_T, while the times to reach the maximum concentration for topotecan lactone and carboxylate were determined graphically. The lactone to total concentration ratio in the 21-day continuous infusion study was calculated as the concentration of the lactone form divided by the concentration of total topotecan.

In vitro experiments

From five male volunteers a volume of 12 ml heparinized blood was collected, from which 6 ml was centrifuged for 5 min at 2000 *g* to separate plasma and blood cells. The plasma supernatants, combined with the buffy-coat, and the remaining red blood cell fractions were collected. Fractions of the whole blood, the red blood cells and the plasma were combined to create different hematocrit values in blood of the same individual, ranging between 0.20 and 0.60 l/l. A volume of 1 ml of these samples was incubated simultaneously with 5 ng/ml of topotecan lactone and 5 ng/ml of topotecan carboxylate for 15 min at 37°C to study the impact of the hematocrit on the lactone to total concentration ratio in the plasma compartment. The blood samples were further processed as described above for the blood samples of the patients. The remaining fractions were used for the determination of hematocrit values. To confirm the gender-related difference in the topotecan pharmacokinetics, 1 ml of normal heparinized whole blood of five female and five male volunteers was incubated with 5 ng/ml of topotecan lactone and carboxylate, and further processed as described above.

Statistical analysis

All parameters are reported as mean values $\pm$ SD. Two-tailed unpaired Student's *t*-tests were performed to evaluate statistically significant differences ($p < 0.05$) in pharmacokinetic and biochemical parameters between males and females, using the NCSS package (version 5.X; JL Hintze, East Kaysville, UT, 1992). Linear regression analysis was performed to test potential relationships between parameters, using the same program.

Results

Clinical pharmacokinetics

A total of 54 patients (36 male and 18 females) enrolled in the oral phase I study were evaluated for pharmacokinetic analysis during day 1 of course 1. Since cisplatin has no effect on the pharmacokinetics

of topotecan,[7] courses without and in combination with i.v. administered cisplatin were used for the determination of gender-dependent differences in topotecan pharmacokinetics. The pharmacokinetic and biochemical characteristics of the evaluable patients are listed in Table 1. The apparent clearance of topotecan lactone was significantly 1.4-fold faster in males as compared to females (p=0.0082), while after correction for the body surface area the apparent clearance of the lactone form was remained significantly 1.3-fold faster in males (p=0.031). Interestingly, no significant differences were observed in the clearance of total topotecan. The lactone to total ratio of the AUC was significantly 1.3-fold higher in females (p=0.0076), and a significant correlation (r=0.35, p=0.0086) was found between the lactone to total AUC ratio and the apparent clearance of topotecan lactone (Figure 2a). Linear regression analysis was performed between, respectively, the significantly different biochemical characteristics body surface area and hematocrit, and the apparent clearance of topotecan, expressed in l/h as well as in l/h/m^2. A significant relationship was found between the body surface area and the absolute apparent clearance expressed in l/h (p=0.013), while no significant relationship was found after correction of the apparent clearance for the body surface area. In contrast, the relationship between hematocrit and the absolute as well the corrected apparent clearance was significantly correlated (p=0.040 and p=0.030, respectively). In the continuous i.v. phase II study, 38 patients (19 males and 19 females) had evaluable topotecan pharmacokinetics on day 8 of course 1, with a 1.2-fold higher lactone to total concentration in females (Table 1). As in the oral study, a significant correlation (r=0.61, $p<0.0001$) between the lactone to total concentration and the apparent clearance of topotecan lactone was found (Figure 2b).

In vitro studies

We sought to define an *in vitro* model that would explain the differences between males and females in topotecan pharmacokinetics. In the experiments with whole blood samples of the healthy male volunteers, with artificially altered hematocrit values, a strong significant correlation (r=0.98, $p<0.0001$) was found between the hematocrit value and the lactone to total

Table 1. Pharmacokinetic and biochemical characteristics

	Males	Females	p
Oral phase I study			
L/T (%)	37.1±5.32 (n=36)	41.7±6.51 (n=18)	0.0076
CL/F_L (l/h)	237±105 (n=36)	163±62.5 (n=18)	0.0082
CL/F_L (l/h/m^2)	123±53.3 (n=36)	92.4±33.3 (n=18)	0.031
CL/F_T (l/h)	85.0±33.3 (n=36)	69.1±29.9 (n=18)	NS
CL/F_T (l/h/m^2)	44.7±17.9 (n=36)	38.5±14.6 (n=18)	NS
T_{maxL} (h)	1.63±1.25 (n=36)	1.92±1.60 (n=18)	NS
T_{maxC} (h)	2.79±1.43 (n=36)	2.96±1.67 (n=18)	NS
$T_{½L}$ (h)	2.97±1.12 (n=36)	3.11±0.912 (n=18)	NS
$T_{½C}$ (h)	3.65±1.05 (n=36)	3.47±0.722 (n=18)	NS
BSA (m^2)	1.96±0.204 (n=36)	1.77±0.204 (n=18)	0.0003
Ht (l/l)	0.39±0037 (n=30)	0.36±0.041 (n=16)	0.015
CL_{creat} (ml/min)	89±20 (n=21)	80±19 (n=10)	NS
albumin (g/l)	41±4.0 (n=34)	41±2.9 (n=15)	NS
total protein (g/l)	76±5.3 (n=34)	75±4.8 (n=15)	NS
21-day continuous i.v. infusion			
L/T (%)	29.6±6.67 (n=18)	34.1±5.70 (n=19)	0.034
CL_L (l/h)	73.9±27.8 (n=19)	59.9±13.6 (n=19)	NS
CL_L (l/h/m^2)	37.5±14.3 (n=19)	34.3±8.14 (n=19)	NS
CL_T (l/h)	21.3±5.78 (n=18)	20.0±3.85 (n=19)	NS
CL_T (l/h/m^2)	10.4±3.88 (n=18)	11.5±2.56 (n=19)	NS
BSA (m^2)	1.98±0.139 (n=19)	1.76±0.164 (n=19)	0.0001
Ht (l/l)	0.37±0.038 (n=12)	0.34±0.028 (n=16)	0.023
CL_{creat} (ml/min)	98±16 (n=9)	87±22 (n=14)	NS
albumin (g/l)	43±4.0 (n=12)	41±6.1 (n=16)	MS
total protein (g/l)	70±8.9 (n=12)	71±16 (n=16)	NS

L/T (%) = lactone to total ratio, CL/F and CL = (apparent) clearance of topotecan lactone and total, T_{max} = time to reach the maximal plasma concentration of topotecan alctone and carboxylate, $T_{½}$ = terminal half-life of topotecan lactone and carboxylate, BSA = body surface area, Ht = hematocrit, CL_{creat} = creatinine clearance, NS = no significant difference.

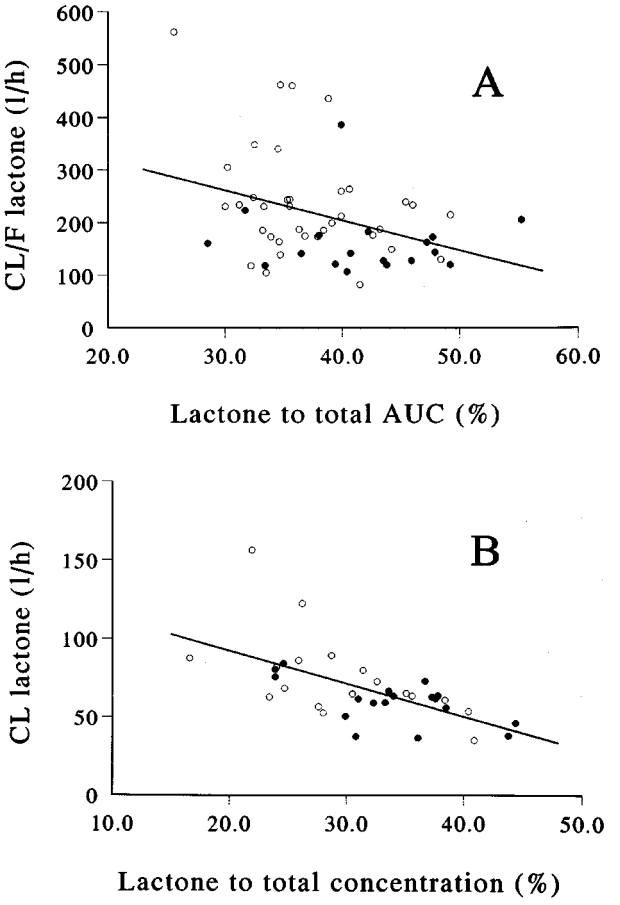


Figure 2. Relationship between the lactone to total ratios and the apparent topotecan lactone clearance in patients treated with oral topotecan (A) or i.v. topotecan (B). Male patients are indicated by open circles and females by closed circles.

concentrations after a 15 min incubation at 37°C with 5 ng/ml of both topotecan lactone and carboxylate (Figure 3). By comparing normal heparinized whole blood samples of five male and five female volunteers, a significant difference (p=0.0015) in the hematocrit value was found, with mean values of 0.44 ± 0.014 l/l for males and 0.39 ± 0.019 l/l for females. After a 15 min incubation at 37°C with 5 ng/ml of topotecan lactone and carboxylate, the lactone to total concentration ratio in the plasma compartment was significantly higher ($p < 0.0001$) in females as compared to males, with respective values of 27.8 ± 0.41 and 25.2 ± 0.36%. In addition, the change in the lactone to total concentration ratio was accompanied by a significantly (p=0.010) higher exposure of the lactone form in the plasma compartment of the blood samples of the female volunteers, with mean concentrations of 3.41 ± 0.103 and 3.22 ± 0.074 ng/ml for the blood samples obtained from the female and male volunteers.

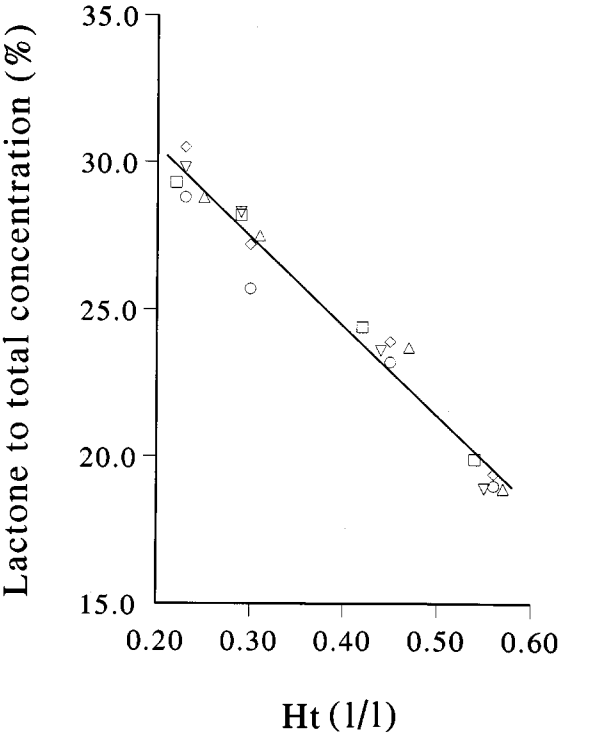


Figure 3. Relationship between the hematocrit (Ht) and the lactone to total concentration ratio in plasma. Each symbol represents the created hematocrit value versus the lactone to total ratio in the plasma of one healthy volunteer.

Discussion

In the present study, we have demonstrated for the first time that topotecan clearance is significantly slower in females as compared to males. These data complement previous knowledge of the clinical pharmacology of topotecan and may have important clinical implications for its optimal use. Previous studies have revealed that major factors responsible for gender-dependent pharmacokinetics are related to differences in body composition, renal elimination, drug absorption and hepatic function.[6] Indeed, a significant difference in the body surface area between male and female patients was found in the present study. However, the apparent clearance of topotecan lactone after oral administration was still 1.3-fold higher in male patients as compared to female patients after correction for body surface area, indicating that body surface area was not the major predictor for the gender-dependent clearance of topotecan lactone. Likewise, no significant linear relationship was found between body surface area and the apparent clearance of topotecan lactone after correction of the apparent clearance for body surface area.

Pharmacokinetic studies performed during previous clinical trials of topotecan have consistently failed to recognize the discrepant drug disposition in males and females. This is most likely caused by the fact that in most studies only limited numbers of patients were sampled or combined measurement of topotecan lactone plus carboxylate was performed. Recently, gender-dependent differences in topotecan pharmacokinetics were not found in a population of children and adults in the range 3 weeks to 22 years of age.[10] This is probably related to the fact that no gender differences in hematocrit values are reported in children under the age of 12 years and only marginal differences were observed at 12–18 years.[11]

Topotecan is mainly eliminated by the kidneys, with 40% (range 26–80%) of the dose excreted in the urine as parent compound within 24 h after a 30-min i.v. infusion.[1] One of the known metabolic pathways of topotecan is the loss of the methyl moiety linked to the nitrogen in the core structure of topotecan by the cytochrome P450 enzyme system, resulting in *N*-desmethyl topotecan (Figure 1). Concentrations of this metabolite in plasma and urine were very low; after a 30-min i.v. infusion, peak plasma concentrations of *N*-desmethyl topotecan accounted for less than 1% of the maximal total drug concentration and in urine only 1–4% of the delivered dose was excreted as *N*-desmethyl topotecan.[12] Recently, a new metabolic conjugation pathway has been described,[13] resulting in the formation of topotecan-*O*-glucuronide and *N*-desmethyl topotecan-*O*-glucuronide (Figure 1). Since relatively low amounts of these metabolites were excreted in the urine, with maximal concentrations of 10 and 3.5%, respectively, in comparison with urinary concentrations of the parent compound, coupled with the fact that altered topotecan clearance only has been described in patients with severely impaired renal function,[14] gender-dependent differences in the known metabolic pathways of topotecan are unlikely to occur. However, minor gender-related differences in renal clearance could be expected, since the glomerular filtration rate of the kidneys is related to the body weight and thus higher topotecan clearance in males could be due to their higher body weight.[6] Nevertheless, as described above, the apparent clearance of topotecan lactone was significantly higher in males, even after correction for the body surface area (i.e. body weight), while no significant difference was found for the clearance of total topotecan.

Differences in intestinal drug absorption between males and females after oral administration have been reported and shown to be possibly related to a slower gastric emptying rate in females, different levels of gut enzymes and differences in the hepatic first-pass effect. In the present analysis, however, differences in the gastric emptying rate are less likely, since the time to reach the maximum concentration of topotecan lactone after oral administration did not differ significantly. Moreover, previously we did not find differences in the oral bioavailability of the i.v. dosing solution between males and females of topotecan lactone, with bioavailabilities of $31 \pm 8.4\%$ (n=7) and $30 \pm 7.5\%$ (n=5) for males and females, respectively (data compiled from Schellens *et al.*[15]).

Gender-related differences caused by different levels of liver and gut enzymes are not expected since metabolism is a minor route of elimination of topotecan. As described above, low amounts of the known metabolites of topotecan were detected in urine and plasma of patients. Likewise, in a phase I and pharmacologic study in patients with impaired hepatic function, similar topotecan pharmacokinetics were observed in patients with and without liver injury,[16] also suggesting a minor role of liver enzymes in the overall elimination of topotecan.

A significant relationship was found following linear regression analysis of body surface area versus the absolute apparent clearance of topotecan lactone, while after correction for body surface area this relationship did not remain statistically significant. However, significant relationships were noted between the hematocrit and the absolute apparent clearance of topotecan lactone, as well as the apparent clearance corrected for body surface area, indicating that hematocrit was a significant predictor for the apparent clearance of topotecan lactone.

To further evaluate the role of hematocrit in topotecan pharmacokinetics as a potentially important contributing factor to the observed gender dependency, various additional *in vitro* studies were performed. Hematocrit values in healthy humans are known to be different in males and females, with, respectively, values of 0.44 ± 0.02 and 0.39 ± 0.02 l/l.[17] Furthermore, erythrocytes are known to be carriers for a variety of endogenous compounds and drugs, including topotecan.[18–20] Drugs and endogenous compounds in the plasma compartment are in equilibrium between plasma proteins and plasma water, i.e. in a bound and unbound form. The plasma water is the central compartment, from which the unbound drug is able to move across cell membranes, including those of red blood cells. Topotecan has a plasma protein binding of approximately 35%[21] and hence 65% of the drug in principle is directly available for cellular uptake. To demonstrate the relationship between hematocrit value and the lactone to total concentration ratio in plasma, whole blood of five male volunteers, with artificially altered hematocrit

values, was incubated with topotecan lactone and carboxylate. The hematocrit appeared to be a principle predictor of the resulting topotecan lactone to total concentration ratio in the plasma compartment, with higher ratios at lower hematocrit values. This phenomenon was confirmed by *in vitro* incubation of topotecan in whole blood of males and females, showing significantly higher lactone to total topotecan concentrations in females as compared to males. The higher lactone to total topotecan ratios in blood with lower hematocrit values is most likely caused by the fact that the carboxylate form, which is charged, is not able to pass cell membranes and thus remains in the plasma compartment.[22] Hence, the absolute amount of the carboxylate form in the plasma compartment in the *in vitro* experiments is independent of the hematocrit value, resulting in lower carboxylate concentrations in blood samples with lower hematocrit values. In addition, we found significantly higher topotecan lactone concentrations in the plasma compartment of the blood samples of the female volunteers. This phenomenon is consistent with the *in vivo* finding of lower topotecan lactone clearance in females, as a result of higher exposure of the lactone form in females compared to males.

In conclusion, we have shown that topotecan is subject to significant gender-dependent differences in pharmacokinetic behavior that result from a physiologic difference in hematocrit values between males and females. This finding may have implications for interpretation of the relationship between pharmacokinetic parameters and pharmacodynamic outcome of topotecan treatment. A potential gender-dependent relationship between the pharmacokinetics and pharmacodynamics has to be investigated in a study using single-agent topotecan at a fixed dose. Eventually, pharmacologic data generated in this investigation and the recognition of the gender dependency in topotecan pharmacokinetics may provide a basis for the development and refinement of clinical protocols allowing more rational and selective treatment with topotecan.

References

1. Herben VMM, ten Bokkel Huinink WW, Beijnen JH. Clinical pharmacokinetics of topotecan. *Clin Pharmacokinet* 1996; **31**: 85–102.
2. Herben VMM, Rosing H, ten Bokkel Huinink WW, *et al*. Oral topotecan: bioavailability and effect of food coadministration. *Br J Cancer* 1999; **80**: 1380–6.
3. Gerrits CJH, Schellens JHM, Burris H, *et al*. A comparison of clinical pharmacodynamics of different administration schedules of oral topotecan. *Clin Cancer Res* 1999; **5**: 69–75.
4. Gore M, Rustin G, Calvert H, *et al*. A multicenter, randomized, phase II study of topotecan (T) administered intravenously or orally for advanced epithelial ovarian carcinoma. *Proc Am Soc Clin Oncol* 1998; **17**: 349a (abstr 1346).
5. Von Pawel J, Gatzemeier U, Harstrick A, *et al*. A multicenter randomized phase II study of oral topotecan versus iv topotecan for second line therapy in sensitive patients with small cell lung cancer. *Proc Am Soc Clin Oncol* 1999; **18**: 471a (abstr 1816).
6. Harris RZ, Benet LZ, Schwartz JB. Gender effects in pharmacokinetics and pharmacodynamics. *Drugs* 1995; **50**: 222–39.
7. de Jonge MJA, Loos WJ, Gelderblom H, *et al*. Phase I and pharmacologic study of oral topotecan and intravenous cisplatin: sequence dependent hematological side-effects. *J Clin Oncol* 2000; **18**: 2104–15.
8. Creemers GJ, Gerrits CJH, Schellens JHM, *et al*. Phase II and pharmacologic study of topotecan administered as a 21-days continuous infusion to patients with colorectal cancer. *J Clin Oncol* 1996; **14**: 2540–5.
9. Loos WJ, Stoter G, Verweij J, Schellens JHM. Sensitive high-performance liquid chromatographic fluorescence assay for the quantitation of topotecan (SKF 104864-A) and its lactone ring-opened product (hydroxy-acid) in human plasma and urine. *J Chromatogr B* 1996; **678**: 309–15.
10. Stewart CF, Liu CY, Zamboni WC, *et al*. Population pharmacokinetics of topotecan in children and adolescents. *Proc Am Soc Clin Oncol* 2000; **19**: 177a (abstr 687).
11. Tietz NW. *Clinical guide to laboratory tests*. Philadelphia, PA: Saunders 1983: 258.
12. Rosing H, Herben VMM, van Gortel-van Zomeren DM, *et al*. Isolation and structural confirmation of *N*-desmethyl topotecan, a metabolite of topotecan. *Cancer Chemother Pharmacol* 1997; **39**: 498–504.
13. Rosing H, van Zomeren DM, Doyle E, Bult A, Beijnen JH. *O*-Glucuronidation, a newly identified metabolic pathway for topotecan and *N*-desmethyl topotecan. *Anti-Cancer Drugs* 1998; **9**: 587–92.
14. O'Reilly S, Rowinsky EK, Slichenmyer W, *et al*. Phase I and pharmacologic study of topotecan in patients with impaired renal function. *J Clin Oncol* 1996; **12**: 3062–73.
15. Schellens JHM, Creemers GJ, Beijnen JH, *et al*. Bioavailability and pharmacokinetics of oral topotecan: a new topoisomerase I inhibitor. *Br J Cancer* 1996; **73**: 1268–71.
16. O'Reilly S, Rowinsky EK, Slichenmyer W, *et al*. Phase I and pharmacologic studies of topotecan in patients with impaired hepatic function. *J Natl Cancer Inst* 1996; **88**: 817–24.
17. Saxena S, Wong ET. Heterogeneity of common hematologic parameters among racial, and gender subgroups. *Arch Pathol Lab Med* 1990; **114**: 715–9.
18. Higley MS, de Bruijn EA. Erythrocytes and the transport of drugs and endogenous compounds. *Pharm Res* 1996; **13**: 186–95.
19. Schrijvers D, Higley MS, de Bruijn EA, van Oosterom AT, Vermorken JB. Role of red blood cells in pharmacokinetics of chemotherapeutic agents. *Anti-Cancer Drugs* 1999; **10**: 147–53.

20. Cole DE, Blaney SM, Balis FM. Topotecan in physiologic fluids: depot of active drug in red blood cells. *Proc Am Ass Cancer Res* 1995; **36**: 363 (abstr 2161).
21. Anonymous. *Hycamtin* (product monograph). Smith-Kline Beecham Pharmaceuticals 1996.
22. Loos WJ, Verweij J, Gelderblom AJ, *et al*. Role of erythrocytes and serum proteins in the kinetic profile of total 9-amino-20-(*S*)-camptothecin in humans. *Anti-Cancer Drugs* 1999; **10**: 705–10.

(Received 13 July 2000; revised form accepted 27 July 2000)