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Pharmacokinetics and pharmacodynamics of topotecan administered daily for 5 days every 3 weeks

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Abstract Topotecan is a novel semisynthetic derivative of the anticancer agent camptothecin and inhibits the intranuclear enzyme topoisomerase I. The lactone structure of topotecan, which is in equilibrium with the inactive ring-opened hydroxy acid, is essential for this activity. The open form predominates at physiological pH. We performed a pharmacokinetic study as part of a phase I study in patients with various types of solid tumors, where topotecan was administered in a 30-min infusion daily on 5 consecutive days every 3 weeks. The plasma kinetics of topotecan could be described best using an open two-compartment model with t½(α) and t½(β) of 8.1 (range 0.3 to 40.7) min and 132 (range 49 to 286) min, respectively. The plasma concentration-time profiles of the metabolite, however, could be described using a one-compartment model with t½(formation) of 29.0 (range 5.6–99.5) min and t½ (elimination of 123.2 (range 32–265) min, respectively. The lactone was the predominate form during the first hour from the start of infusion, but was rapidly converted into its ring-opened structure. The elimination rate of topotecan was independent of the dose. There were linear relationships between the dose (mg m^{-2} day^{-1}), the area under the plasma concentration versus time curve (AUC) of topotecan and its metabolite, the total AUC, peak plasma lactone concentrations, and the time period that the topotecan concentrations remained above 10 n*M*. Different models were used to correlate pharmacokinetic and pharmacodynamic parameters. The percentage decrease in absolute neutrophil count (ANC) was related to these parameters and plots were well fitted by linear and sigmoidal E_{max} models.

Key words Pharmacodynamics · Pharmacokinetics
Topotecan · Topoisomerase I inhibitor

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Introduction

Topotecan ([S]-9-dimethylaminomethyl-10-hydroxy-camptothecin hydrochloride, SK&F 104864-A, NSC 609699), is a novel semisynthetic derivative of camptothecin, an anticancer drug derived from the Asian tree *Camptotheca acuminata*. Owing to camptothecin's serious and unpredictable gastrointestinal, urothelial and myelosuppressive toxicities, clinical evaluation had to be discontinued in the early 1970s [18, 19]. Compared with campthothecin, topotecan is more water soluble, has reduced protein binding, and shows promising efficacy with a strongly reduced toxicity profile [2, 6, 11, 16, 22, 33]. The dose-limiting toxicity of topotecan is reversible myelotoxicity, especially granulocytopenia [22, 23, 26, 30, 33], and mucositis [16].

Topotecan and other camptothecin analogues inhibit the intranuclear enzyme topoisomerase I. Topoisomerase I is involved in RNA transcription, DNA replication, and possibly DNA repair and genetic rearrangements [1, 13, 27]. During the process of winding and unwinding of DNA, torsional stresses and topological problems occur. Topoisomerase enzymes change the conformation of a segment of DNA, resolving these mechanical obstacles [1]. Topotecan and other camptothecin analogues prohibit RNA transcription by stabilizing the DNA-topoisomerase I "cleavable complexes," which can result in lethal DNA damage during the courses of DNA replication [10, 13]. The lactone structure, which is in equilibrium with the open-ring hydroxy acid (SK&F 105992) at constant pH is essential for this function (Fig. 1) [11, 31]. The closed lactone ring predominates at acidic pH, but the reverse reaction of the parent into the metabolite predominates at physiological pH

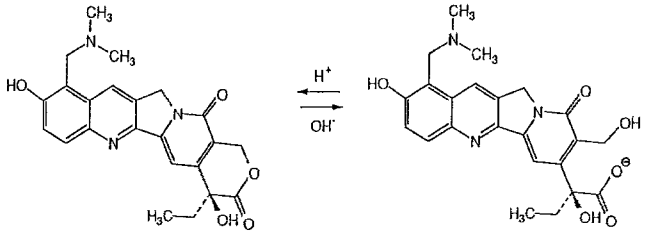

Fig. 1 A, B Chemical structures of **A** topotecan and **B** its lactone ring-opened hydroxy acid. Both forms are in equilibrium at constant pH

[2, 28]. The spontaneous hydrolysis of the parent drug to the inactive form may, therefore, have important pharmacokinetic and pharmacodynamic implications.

Although neutropenia and leukopenia were correlated to pharmacokinetic parameters, such as the AUC and peak plasma (C_{max}) concentrations of topotecan in previous studies, only weak or absent relations were found [3–5, 32, 33].

We performed a pharmacokinetic study as part of a phase I study [30], using a high-performance liquid chromatography [HPLC] procedure developed in our laboratory [2], by which we can measure selectively and simultaneously both topotecan and its lactone ring-opened structure. The pharmacokinetic data have been correlated with the pharmacodynamic outcome of the phase I study by using different mathematical models.

Patients and methods

Patient population

The patients, from whom pharmacokinetic curves and clinical history were obtained, participated in a phase I trial of topotecan administered daily for 5 consecutive days every 3 weeks [30]. Eligibility criteria included a histologically confirmed diagnosis of a solid malignant tumor no longer amenable to established forms of treatment. All patients had an acceptable bone marrow function (white blood cells (WBC) $>4 \times 10^9$/l and platelets $\geq 100 \times 10^9$/l), serum bilirubin ≤ 26 μM and serum creatinine ≤ 140 μM, with no prior history of hemorrhagic cystitis, WHO performance status ≤ 2, life expectancy of ≥ 12 weeks and age 18–75 years. All patients gave informed consent.

Treatment plan

Topotecan (SmithKline Beecham, King of Prussia, USA) was supplied as a clear yellow solution, containing the hydrochloride salt. This solution consisted of 2.57 mg of topotecan hydrochloride, equivalent to 2 mg of the free base, 52.1 mg of D-gluconic aicd as its monopotassium salt, hydrochloric acid or sodium hydroxide for adjustment to pH 3.0, and water for injection USP q. s. to give 2.2 ml. The appropriate dosage of the drug was diluted in 48 ml of normal saline and administered i. v. by an infusion pump over 30 min on 5 consecutive days every 3 weeks. Dose escalation was performed according to a modified Fibonacci scheme, using doses of 0.5, 0.65, 0.9, 1, 1.25, and 1.5 mg m^{-2} day^{-1}, respectively. Intrapatient dose escalation was not permitted. To determine the hematological and non-hematological toxicities, patients were evaluated weekly by clinical history, physical examination, serum chemistry and hematology screening. At the highest dose level hematology screening was performed twice a week.

Pharmacokinetic studies

Blood samples (5 ml each), taken from an indwelling i. v. cannula placed in the arm contralateral to the arm receiving topotecan, were collected in heparinized tubes at 12 time points: before each infusion, at 5, 10, 15 and 30 min during the infusion, and at 15 and 30 min and 1, 2, 3, 4, and 6 h after the end of the 30-min infusion. Samples were collected during the first and, if possible, during the 4th or 5th day of drug administration. Plasma was obtained by immediate centrifugation (5 min; 1500 *g*) of the samples, followed by protein precipitation with cold methanol: 1 ml plasma was added to 4 ml of methanol (–30° C). Thereafter, the substance was mixed and centrifuged (5 min; 1500 *g*), and the clear supernatant was transferred to a glass autosampler vial and stored (–30° C) until analysis. Topotecan and its lactone ring-opened form were determined by a validated HPLC method using fluorescence detection, developed in our laboratory [2]. Using this method both forms are measured selectively and simultaneously in a single run.

The plasma concentration [C(t)] versus time (t) curves of topotecan were analyzed using the pharmacokinetic software package MW/Pharm (MEDI\WARE, Groningen, The Netherlands) [21]. This non-linear least-squares, iterative regression program determines slopes and intercepts of the logarithmically plotted curves of multi-exponential functions. Initial estimates of the parameters were determined by an automated curve stripping procedure. In general, the mathematical equation describing plasma drug concentration $C(t)$ at any time t during and after i. v. infusion is given during infusion by:

$$C(t) = \sum_{i=1}^{N} \{C_i/(\lambda_i \times T_{inf}) \times (1 - e^{(-\lambda_i \times t)})\} \quad \text{(Eq.1)}$$

and post infusion by:

$$C(t) = \sum_{i=1}^{N} \{C_i/(\lambda_i \times T_{inf}) \times (e^{(-\lambda_i \times |t - T_{inf}|)} - e^{(-\lambda_i \times t)})\} \quad \text{(Eq.2)}$$

where λ_i is the exponent of the i-th exponential term, C_i is the initial concentration of the i-th component of the curve and T_{inf} is the infusion time.

Topotecan kinetics could be best described by a bi-exponential model ($N = 2$), which gave the lowest Akaike information criterium. Curve fitting with this model yields the parameters C_1, C_2, λ_1 and λ_2. Respective half-lives were calculated from the equations $t\frac{1}{2}(\alpha) = 0.693/\lambda_1$ and $t\frac{1}{2}(\beta) = 0.693/\lambda_2$. The area under the curve (AUC) was determined on the basis of the fitted curve as the exact integral of the $C(t)$ versus t plots $(_0\int^{\infty} C(t)dt)$ from $t = 0$ to infinity. Total plasma clearance (CL) was calculated by dividing the dose by the AUC. The computer program also calculated the apparent first-order elimination rate constant from the central compartment (k_{10}), the intercompartmental transfer rate constants k_{12} and k_{21}, the apparent distribution volume of the central compartment (V_c), and the apparent distribution volume during steady state (V_{ss}) from C_1, C_2, λ_1 and λ_2 with standard equations [7]. The pharmacokinetics of the topotecan metabolite were modelled by a bi-exponential equation describing both the formation and elimination of the metabolite in a one-compartment model:

$$C_m(t) = C(e^{(-\lambda_e \times t)} - e^{(-\lambda_f \times t)}) \quad \text{(Eq.3)}$$

where $C_m(t)$ is the metabolite concentration at time t, λ_e and λ_f are apparent first-order rate constants for metabolite elimination and formation, respectively. C is a constant that depends on the values of λ_e, λ_f, the apparent volume of distribution of the metabolite (V_m), the dose of topotecan and f_m, the fraction of drug converted into the metabolite. Half-lives were calculated from the equations: $t\frac{1}{2}(1) = 0.693/\lambda_e$ and $t\frac{1}{2}(2) = 0.693/\lambda_f$. Other pharmacokinetic parameters were calculated with standard equations in the same manner as for topotecan [7].

The peak plasma concentrations (C_{max}) of topotecan and its ring-opened form and the time to reach the maximal concentration (T_{max}) are observed experimental values.

Pharmacokinetic-pharmacodynamic relationships

The pharmacodynamics, especially the dose-limiting toxicities, were explored using plots of percentage decrease (%decr) in WBC and ANC versus the dose (mg m^{-2} day^{-1}) and pharmacokinetic parameters. Since it has previously been suggested that the time period that the topotecan concentration is above 1 or 10 n*M*, among other parameters, can be important for toxicity [9], we investigated the following pharmacokinetic parameters: the C_{max} (n*M*), the time period that the topotecan concentration is above 1.0 n*M* or 10 n*M* topotecan (h), and the AUC (μM·min) of topotecan, its metabolite, and the sum of both.
The percentage decrease (%decr) is defined as:

$$\%\text{decr} = \frac{\text{Pretreatment value} - \text{value of the nadir}}{\text{Pretreatment value}} \times 100\% \quad \text{(Eq.4)}$$

The data were fitted using a (log-) linear model, a maximum effect (E_{max}) model and a sigmoidal maximum effect (sigE_{max}) model, as described by a modified Hill equation [15, 22].
The linear models were:

$$\%\text{decr} = a \cdot (P) + b \quad \text{(Eq.5)}$$

$$\log(\%\text{decr}) = a \cdot (P) + b \quad \text{(Eq.6)}$$

The E_{max} model was:

$$\%\text{decr} = \frac{(ME)(P)}{(P_{50}) + (P)} \quad \text{(Eq.7)}$$

The sigmoidal E_{max} model was:

$$\%\text{decr} = \frac{(ME)(P)^H}{(P_{50})^H + (P)^H} \quad \text{(Eq.8)}$$

Where *a* denotes the slope and *b* the intercept of the linear models, *ME* denotes the maximal effect, which is 100 (i.e. 100% decrease), *P* denotes the value of the pharmacokinetic parameter of interest, *P*50 the *P* that results in 50% of the *ME*, and *H* denotes the Hill constant, which describes the shape of the curve. In the E_{max} model the Hill constant is 1. Linear regression analysis was performed to obtain a correlation coefficient (*r*) and the slope and intercept of the linear model. The computer program NCSS (Number Cruncher Statistical System, Kaysville, Utah, USA, 1992) and Quattro Pro (Borland International, Scotts Valley, Calif., USA, 1992) were used for all calculations.

The performance of the model was evaluated by using the relative root mean square error (%RMSE) value and its standard error (SE) [25]. The %RMSE is a measure of precision and is defined as:

$$\text{RMSE}\% \left[N^{-1} \cdot \sum_{i=1}^{N} (pe_i)^2 \right]^{1/2} \cdot 100\%$$

and the relative SE is defined as:

$$\text{SE}\% \left[(N \cdot (N-1))^{-1} \cdot \sum_{i=1}^{N} ((pe_i)^2 - \text{RMSE})^2 \right]^{1/2} \cdot 100\%$$

where *N* is e.g. the number of *P*-pairs (i.e., true with predicted values), and *pe* is the prediction error [$\ln(P_{\text{true value}}) - \ln(P_{\text{predicted}})$]. The smaller the %RMSE, the better the relation is described by the model. In the most predictive model the RMSE% approaches zero.

Results

Pharmacokinetics

A total of 48 patients, with a variety of malignant solid tumors were entered in the original phase I study [30]. Median age was 56 years (range 25–75), median performance status 1 (range 0–2); most patients were pretreated (75%). Complete plasma concentration time plots were obtained from 19 patients, and from 15 both on day 1 and on day 4 or 5. All patients were sampled during the first course. Figure 2 depicts the average plasma concentration-time curves for topotecan and its ring-opened form at a dose of 1.5 mg/m^2; the shapes of curves at other dose were similar.

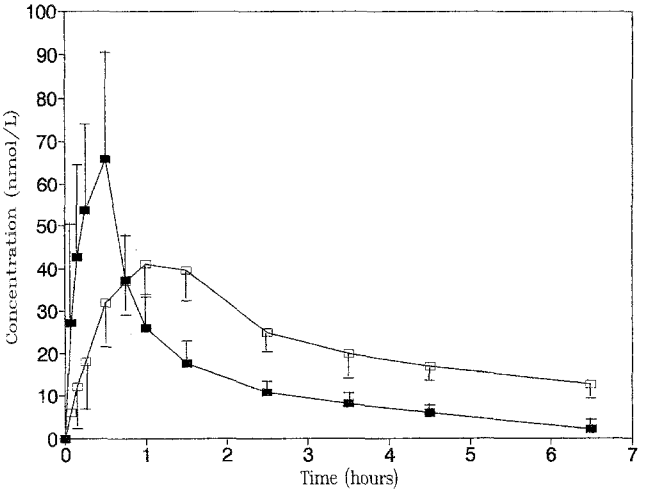

Fig. 2 Average plasma concentration-time curves of topotecan (■) and the lactone ring-opened metabolite (□) with their standard deviation at a dosage of 1.5 mg m^{-2} day^{-1}

The pharmacokinetics of topotecan could be described best with an open two-compartment model. The pharmacokinetic parameters obtained by means of this model are presented in Table 1.

After cessation of the infusion, the topotecan curves exhibited a bi-exponential decay. The mean values and ranges (n = 34) for the linear segments were $t_{1/2}(\alpha)$: 8.1 min (range 0.3–40.7 min) and $t_{1/2}(\beta)$: 132 min (range 49–286 min). The mean values for the compartmental rate constants were: k_{10}: 6.6 (range 0.75–66.6) × 10^{-2} min^{-1}, k_{12}: 19.5 (range 0.62–179.5) × 10^{-2} min^{-1} and k_{21}: 1.81 (range 0.51–6.0) × 10^{-2} min^{-1}. The mean ± SD for the ratio k_{21}/k_{12} was 0.32 ± 0.24. The apparent distribution volumes of topotecan were V_c: 17.0 (range 0.8 to 57.7) l/m^2, and apparent distribution volume at steady state, V_{ss}: 72.7 (range 28.5–123.5) l/m^2. The ratio λ_2/k_{10} was 0.17 ± 0.13. The mean total body clearance of topotecan was 0.57 (range 0.25–0.99) l min^{-1} m^{-2}. Concentrations above 10 n*M* were maintained for a median of 1.8 h (range 0.8–3.2 h). The mean percentage of total drug excreted in the urine over the first 24 h on the 1st day of the 5-day course was 25.8% (range 7.0–58.6%). Peak plasma concentrations of topotecan were reached at the end of the 30-min infusion and ranged between 24.8 and 169.8 n*M*. Most pharmacokinetic parameters failed to show dose-related trends (r < 0.25), exceptions being C_{max} (r = 0.44), the AUC of topotecan (r = 0.66), and the duration of concentrations over 10 n*M* (r = 0.78).

In the first plasma samples the topotecan ring-opened form was already detectable. About 1 h after the start of the

Table 1 Pharmacokinetics of topotecan. (*Pat.* patient number with the day of the course in round brackets; *Dose* mg m^{-2} day^{-1}; C_{max} nM; *AUC* μM · min; $t½(\alpha)$, $t½(\beta)$ min; k_{10}, k_{12}, k_{21} 10^{-2} min^{-1}.; V_c, V_{ss} l/m^2; *CL* l min^{-1} m^{-2}; *t > 10 nM* time (h) above a concentration of 10 nM; $U_{excr.}$ total drug urinary excretion over the first 24 h, given as percentage of the administered total dose on 1 day; *n.d.* not done)

Pat.	Dose	C_{max}	AUC	$t½(\alpha)$	$t½(\beta)$	k_{10}	k_{12}	k_{21}	V_c	V_{ss}	CL	t > 10 nM	$U_{excr.}$
1(1)	0.5	45.2	2.03	4.8	69	7.20	6.11	2.01	7.4	30.1	0.53	0.85	23.6
1(4)	0.5	38.6	2.74	1.9	76	13.40	22.38	2.55	3.0	29.2	0.40	1.0	n.d.
2(1)	0.5	24.3	2.71	7.6	133	2.76	5.15	1.72	14.6	58.1	0.41	1.2	33.9
2(5)	0.5	27.6	2.99	1.2	149	8.02	45.95	3.29	4.6	68.3	0.37	1.0	n.d.
Mean		**33.9**	**2.62**	**3.9**	**107**	**7.85**	**19.90**	**2.39**	**7.40**	**46.4**	**0.43**	**1.0**	**28.8**
3(1)	0.65	22.8	2.10	8.5	167	3.00	4.40	1.11	23.1	114.0	0.69	0.75	13.8
3(4)	0.65	23.7	2.48	11.3	203	2.08	3.39	1.00	28.2	123.5	0.59	0.90	n.d.
4(1)	0.65	42.7	3.68	5.0	154	4.77	8.13	1.29	8.18	58.9	0.39	1.4	10.6
4(4)	0.65	32.6	1.91	12.9	126	2.68	2.15	1.10	27.6	81.7	0.74	1.1	n.d.
Mean		**30.5**	**2.54**	**9.4**	**163**	**3.13**	**4.52**	**1.13**	**21.8**	**94.5**	**0.60**	**1.0**	**12.2**
5(1)	0.9	51.0	3.85	1.1	89	17.80	43.10	2.76	2.9	47.7	0.51	1.5	29.1
5(4)	0.9	55.1	3.72	6.0	117	5.39	5.48	1.27	9.8	52.1	0.53	1.3	n.d.
6(1)	0.9	59.8	3.17	4.0	49	7.54	8.04	3.26	8.2	28.5	0.62	1.4	38.0
7(1)	0.9	42.7	2.96	8.6	132	4.08	3.47	1.04	19.4	84.0	0.79	1.4	23.3
7(4)	0.9	39.7	3.09	5.0	132	5.27	7.65	1.37	4.6	30.2	0.25	1.5	n.d.
8(1)	0.9	45.0	2.49	15.5	139	1.56	1.97	1.44	42.6	101.2	0.66	1.0	n.d.
9(1)	0.9	156.1	8.15	8.6	99	3.06	3.89	1.87	21.0	64.8	0.64	2.9	n.d.
Mean		**64.2**	**3.92**	**7.0**	**108**	**6.39**	**10.5**	**1.85**	**15.5**	**58.4**	**0.57**	**1.8**	**30.1**
10(1)	1.0	43.8	2.87	12.7	192	2.67	2.41	0.74	28.4	121.1	0.76	1.1	24.9
10(4)	1.0	98.2	7.39	6.4	129	3.63	6.07	1.59	8.1	39.1	0.30	2.8	n.d.
Mean		**71.0**	**5.13**	**9.6**	**161**	**3.15**	**4.24**	**1.17**	**18.3**	**80.1**	**0.53**	**2.0**	**24.9**
11(1)	1.25	67.0	5.12	0.3	94	66.6	171.8	2.66	0.8	52.4	0.54	2.2	30.8
11(4)	1.25	90.4	6.05	5.8	109	5.2	5.88	1.46	8.7	43.8	0.45	2.3	n.d.
12(1)	1.25	27.2	2.74	1.0	73	11.1	53.9	6.00	8.9	89.1	0.99	1.6	24.1
12(4)	1.25	44.2	3.81	9.3	93	2.14	3.50	2.59	33.2	78.1	0.71	2.0	n.d.
13(1)	1.25	51.7	4.32	5.4	133	5.61	6.57	1.19	11.4	74.1	0.64	1.4	58.6
13(5)	1.25	90.8	4.18	5.9	125	4.85	6.09	1.35	13.6	75.2	0.66	1.5	n.d.
14(1)	1.25	43.6	4.18	6.3	109	3.80	5.98	1.83	17.2	73.4	0.66	1.8	21.0
14(4)	1.25	52.3	4.02	4.2	111	5.12	9.91	2.01	13.3	78.7	0.68	1.8	n.d.
Mean		**58.4**	**4.30**	**4.8**	**106**	**13.0**	**32.9**	**2.39**	**13.4**	**70.6**	**0.67**	**1.8**	**33.6**
15(1)	1.5	99.0	9.08	21.6	168	1.45	1.26	0.91	27.0	64.3	0.39	3.2	32.2
15(4)	1.5	92.6	8.39	40.7	286	0.81	0.62	0.51	42.6	95.0	0.35	3.0	n.d.
16(1)	1.5	100.1	9.45	15.5	228	1.74	2.26	0.77	27.6	108.0	0.48	2.7	20.2
16(4)	1.5	53.3	6.81	8.5	177	2.51	4.77	1.27	20.4	97.0	0.51	2.4	n.d.
17(1)	1.5	53.0	6.40	2.9	126	7.66	15.4	1.74	6.9	68.1	0.53	2.8	7.0
17(4)	1.5	81.9	6.31	0.3	133	0.75	179.5	1.78	0.8	83.0	0.61	2.5	n.d.
18(1)	1.5	83.4	5.47	15.5	124	1.39	1.83	1.80	57.7	116.0	0.80	2.2	22.3
18(4)	1.5	34.9	4.15	5.1	77	4.15	7.37	2.94	18.4	64.7	0.76	2.3	n.d.
19(1)	1.5	62.2	4.34	6.3	156	3.71	6.49	1.33	9.5	79.2	0.35	2.7	n.d.
Mean		**73.4**	**6.71**	**12.9**	**164**	**2.69**	**24.4**	**1.45**	**23.4**	**86.1**	**0.53**	**2.6**	**20.4**

infusion the concentration exceeded the topotecan concentration and declined in parallel with topotecan (Fig. 2). The pharmacokinetic profiles of the lactone ring-opened form of topotecan could be modelled by Eq. (3), which described both the rates of formation and the elimination of the metabolite in a one-compartment model. The model involved constant rate drug formation and elimination both restricted to one compartment. The pharmacokinetic characteristics of the metabolite are summarized in Table 2. Maximal plasma concentrations were reached at t = 47.6 (range 21–102) min, i.e. about 20 min after the end of the infusion. The mean half-life $t½(2)$, calculated from the terminal linear phase of the metabolite curve, was 123.2 (range 32–265) min and $t½(1)$, the mean half-life of the initial phase of the curve, was 29.0 (range 5.6–99.5) min. The apparent distribution volume of the metabolite was 15.3 (range 1.9–64.5) l/m^2 and the mean total clearance was 0.4 (range 0.17–1.57) l min^{-1} m^{-2}. Linear regression analysis of C_{max} and AUC of the metabolite versus the dose (mg m^{-2} day^{-1}) of topotecan shows relationships with r = 0.66 and r = 0.75, respectively. Linear regression analysis of the total AUC (lactone + hydroxy acid) versus the dose (mg m^{-2} day^{-1}) of topotecan shows a linear relationship with r = 0.78. The other parameters show no dose-related trends. There is, however, a linear relationship between the AUC of topotecan and the AUC of its lactone ring-opened form (r = 0.69).

Figure 3 shows the mean ratio between the concentration of the parent and ring-opened metabolite (lactone/hydroxy acid) as a function of time with its 95% confidence interval.

Table 2 Pharmacokinetics of topotecan's metabolite. (*Pat.* patient number with the day of course in round brackets; *Dose* mg m^{-2} day^{-1}; C_{max} (nM) reached at time T_{max} (min); *AUC* μM · min; $t½(1)$, $t½(2)$ min; f_m fraction of topotecan converted into the metabolite; V_m/f_m l/m^2; CL/f_m l min^{-1} m^{-2})

Pat.	Dose	C_{max}	T_{max}	AUC	$t½(1)$	$t½(2)$	V_m/f_m	CL/f_m
1(1)	0.5	19.8	21	1.26	10.7	37	13.4	0.87
1(4)	0.5	8.0	31	2.52	10.2	113	6.3	0.43
2(1)	0.5	9.42	31	0.69	28.5	32	64.5	1.57
2(5)	0.5	11.5	45	2.25	5.6	186	3.9	0.48
Mean		**12.2**	**32**	**1.68**	**13.8**	**92**	**22.0**	**0.84**
3(1)	0.65	10.1	102	3.10	33.0	126	22.0	0.47
3(4)	0.65	9.7	45	2.44	27.8	107	23.7	0.59
4(1)	0.65	18.2	89	7.07	61.1	94	17.6	0.20
4(4)	0.65	17.0	93	2.82	50.4	55	36.3	0.50
Mean		**13.8**	**82**	**3.86**	**43.1**	**96**	**24.9**	**0.44**
5(1)	0.9	20.2	46	4.74	12.7	134	7.5	0.41
5(4)	0.9	20.0	31	3.85	12.2	125	9.0	0.51
6(1)	0.9	21.5	60	6.18	27.1	130	12.5	0.32
7(1)	0.9	45.6	30	7.93	20.7	63	21.3	0.71
7(4)	0.9	24.5	60	6.98	7.8	144	1.9	0.17
8(1)	0.9	26.6	30	2.79	34.2	108	12.3	0.25
9(1)	0.9	69.7	30	11.54	22.4	141	9.4	0.29
Mean		**32.6**	**41**	**6.29**	**19.5**	**121**	**10.6**	**0.38**
10(1)	1.0	14.3	45	3.31	32.9	98	31.3	0.66
10(4)	1.0	34.5	45	7.45	19.0	118	7.9	0.29
Mean		**24.4**	**45**	**5.38**	**26.0**	**108**	**19.6**	**0.48**
11(1)	1.25	39.0	90	16.2	8.5	265	2.1	0.17
11(4)	1.25	41.8	30	13.6	19.7	180	5.7	0.20
12(1)	1.25	29.7	60	6.07	8.9	137	5.8	0.45
12(4)	1.25	27.1	30	5.42	32.0	81	23.0	0.50
13(1)	1.25	22.6	35	4.13	16.7	100	16.2	0.67
13(5)	1.25	24.8	30	4.60	23.3	111	20.1	0.60
14(1)	1.25	18.2	36	4.24	22.6	109	20.8	0.64
14(4)	1.25	17.3	31	4.69	18.6	131	15.6	0.58
Mean		**27.6**	**43**	**7.37**	**18.8**	**139**	**13.7**	**0.48**
15(1)	1.5	48.4	30	11.3	64.8	76	16.8	0.36
15(4)	1.5	38.7	42	9.16	99.5	118	18.7	0.26
16(1)	1.5	47.8	35	12.8	41.9	161	15.7	0.26
16(4)	1.5	37.9	50	12.7	64.7	90	30.8	0.33
17(1)	1.5	35.2	32	9.98	15.3	203	4.2	0.19
17(4)	1.5	47.6	45	17.1	9.2	179	3.0	0.23
18(1)	1.5	56.7	30	14.4	85.0	96	13.3	0.25
18(4)	1.5	49.9	90	13.3	28.0	184	4.2	0.24
19(1)	1.5	42.0	90	13.4	9.8	156	4.0	0.28
Mean		**44.9**	**49**	**12.7**	**46.4**	**140**	**12.3**	**0.27**

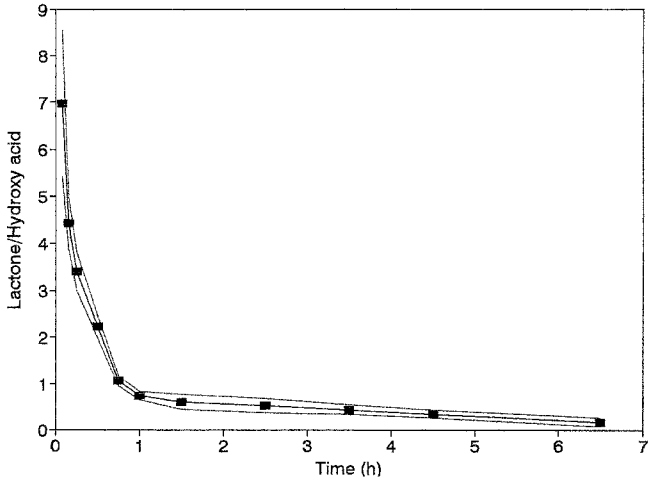

Fig. 3 Mean ratio between plasma concentrations of topotecan and its ring-opened metabolite (lactone/hydroxy acid) as function of time, with its 95% confidence interval

The ratio declines rapidly from approximately 7 to 1, followed by a slow decrease to about 0.17 at 6 h after the start of infusion.

The pharmacokinetic parameters calculated from the analysis on day 1 for each patient are comparable with the values obtained on day 4 or 5 for the same patient (paired Student's *t*-test; $P < 0.94$). However, in two patients (4 and 10; Tables 1, 2) large differences in AUC values were observed.

Hematological toxicity

In all 48 patients the main toxicity was myelosuppression, especially granulocytopenia, with 56% of the evaluable courses resulting in grade 4 granulocytopenia. Thrombocytopenia was much less frequent and less severe (mean nadir $125 \pm 87 \times 10^9$/l). Anemia occurred regularly but was only incidentally severe (average decrease of 16% ± 6%). The

Table 3 Precision of models correlating (log-) %decr in ANC versus the dose and pharmacokinetic parameters. The precision is evaluated by using the percentage root mean square error (%RMSE) value and its standard error (SE). The smaller the %RMSE, the better the relation is described by the model (*P* the pharmacokinetic parameter of interest; *r* correlation coefficient)

Model:	Linear			Loglinear			E_{max}		$sigmE_{max}$	
%decrANC =	$a \cdot P + b$			$\log(\%decr) = a \cdot P + b$			$\frac{(ME)(P)}{(P_{50}) + (P)}$		$\frac{(ME)(P)^H}{(P_{50})^H + (P)^H}$	
P			*r*			*r*				
Dose	11.2	(2.1)	.90	11.4	(2.2)	.89	21.5	(3.7)	13.8	(2.6)
C_{max}	20.0	(3.5)	.61	20.1	(3.5)	.61	27.1	(4.6)	18.8	(3.4)
Time above 1.0 n*M*	25.5	(4.4)	.04	25.3	(4.3)	.07	30.1	(4.6)	38.6	(6.7)
Time above 10 n*M*	13.5	(2.5)	.84	14.5	(2.7)	.82	22.7	(3.8)	13.0	(2.5)
AUC of lactone	20.1	(3.5)	.61	20.4	(3.5)	.59	25.5	(4.2)	17.5	(3.2)
AUC of hydroxy acid	15.6	(2.9)	.81	16.1	(2.9)	.77	28.5	(6.2)	18.8	(3.6)
Total AUC	15.1	(2.8)	.81	15.8	(2.9)	.78	21.5	(3.7)	13.6	(2.6)

nadirs of both leukocytopenia and granulocytopenia were between day 8 and 15 and were of brief duration (3–5 days). Previous chemo- and/or radiotherapy could not be identified as risk factors. There were no indications of cumulative myelotoxicity. In contrast, the extent of myelosuppression during the first course of treatment appeared to be repetitive in all subsequent courses.

Non-hematological toxicity

Nausea and vomiting were found to be non-dose-dependent and occurred in 49 courses (23%), being classed as grade 2 or 3 in 29 courses (13%). They were easily prevented with standard antiemetics in most patients in subsequent courses. Alopecia was also not dose-dependent and occurred in 9 patients (19%), being total in 5 (10%). Other incidental side effects were asymptomatic hypotension (35 courses, 16%), mild proteinuria (8 courses, 4%), and microscopic hematuria (6 courses, 3%). There was no gastrointestinal, cardiac, liver, renal, or skin toxicity.

Responses

Response could be evaluated in 40 patients. A partial remission was seen in 3 female patients (1 with small-cell lung cancer, 1 with non-small-cell lung cancer, and 1 with metastatic pancreatic cancer with a partial remission of the liver metastases). Unfortunately, these patients did not participate in our pharmacokinetic studies. Stable disease was seen in 24 patients.

Modelling pharmacokinetic-pharmacodynamic relationships

The pharmacokinetic parameters obtained from 19 patients (i.e. the C_{max}, the time period above a concentration of 1.0 n*M* or 10 n*M* and the AUC of topotecan, the AUC of its metabolite, the total AUC, and the dose), were plotted against the percentage decrease in WBC and in ANC. Plots of the percentage decrease in ANC and in WBC versus other pharmacokinetic parameters did not yield any significant relationship.

We compared four different models for their ability to describe the data. All data were modelled using a linear, a log-linear, an E_{max} and a sigmoidal E_{max} model [Eqs. (5), (6), (7), (8), respectively]. In general, the decrease (%) in ANC, which was dose-limiting, showed better correlations than that in WBC (data not shown). All models predicting the decrease (%) in WBC were more or less equally predictive: all RMSE% values were greater than 19.3% (median 21.6%, ranging to 54.8%), and all correlation coefficients were smaller than 0.68. The decrease (%) in ANC appeared to be best related to the dose (mg m^{-2} day^{-1}), the total AUC, and the time period above > 10 n*M* (Table 3, Figs. 4–6). All models and comparable precision. Using the $sigmE_{max}$ model, the estimate for the dosage associated with 50% decrease in ANC is 0.6 mg m^{-2} day^{-1}. A dosage of 1.5 mg m^{-2} day^{-1} for 5 consecutive days was considered the maximum tolerable dose (MTD) and recommended for phase II studies [30].

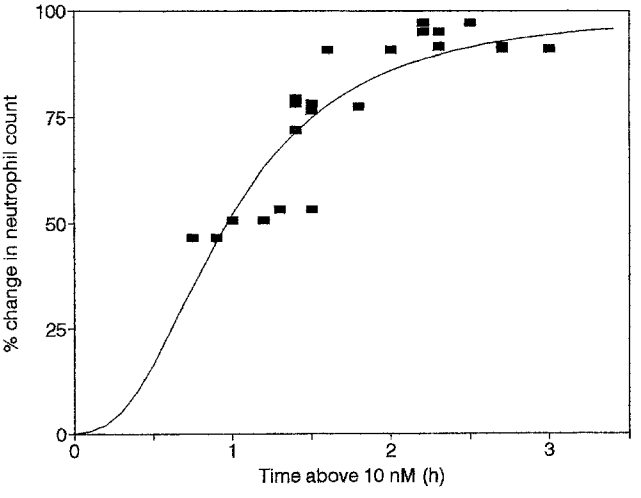

Fig. 4 Relation between the time above a concentration of 10 n*M* of topotecan and the decrease (%) in ANC. The *solid line* represents the fit of the data to a $sigE_{max}$ model

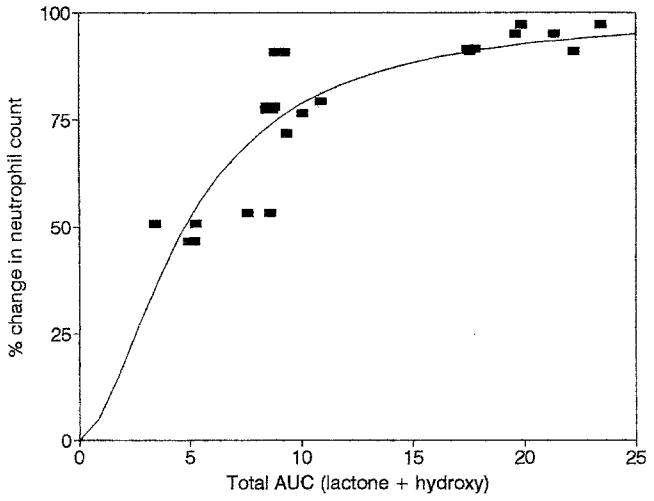

Fig. 5 Relation between the total AUC (lactone + hydroxy) and the %decr in ANC. The *solid line* represents the fit of the data ot a sigE_{max} model

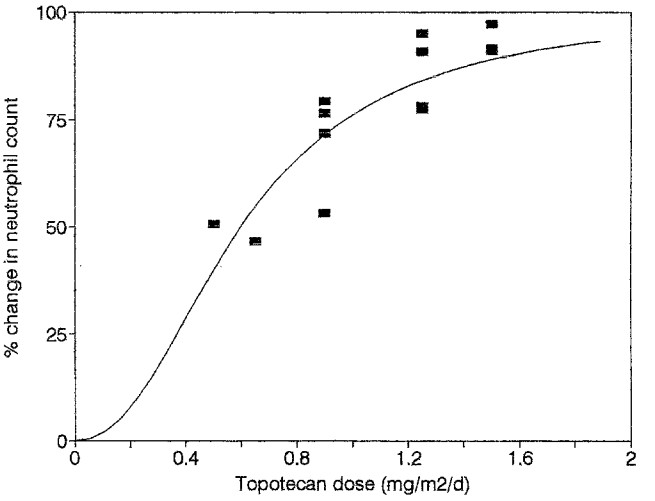

Fig. 6 Relation between the dose (mg/m^2/day) and the %decr in ANC. The *solid line* represents the fit of the data to a sigE_{max} model

Discussion

The chemical conversion of topotecan into the ring-opened species appeared from the start of infusion and continued until the last measured concentration (Fig. 3). The highly sensitive HPLC assay (detection limit $\approx$ 0.4 n*M*) allowed selective measurement of topotecan and its ring-opened form [2]. Therefore, it was possible to construct a complete pharmacokinetic curve even at the first dose step of the study. In plasma at 37° C, no significant amount of the reverse reaction of the ring-opened form into topotecan occurs [2] and, therefore, the reversibility of the reaction has not been taken into account in the pharmacokinetic modelling. The experimental data fitted well to the models used (Eqs. 1–3), indicating that this is justified. Unlike camptothecin, topotecan does not produce hemorrhagic cystitis, despite a 26% drug excretion in the urine over the first 24 h, and it has a reproducible and reduced toxicity profile. Compared with the 17% of camptothecin that was found in the urine after 48 h [8], the occurrence of hemorrhagic cystitis is most likely to be related to the improved water solubility and not the extent of urinary excretion.

The median ratio k_{21}/k_{12}, reflecting the return and entry of topotecan from the peripheral compartment, is 0.32, indicating the tendency of the drug to stay behind in this compartment. Extensive binding to tissues and other components of this compartment may occur, which retards the return into the central compartment. Although this conclusion is speculative, the high value for V_{ss} is in agreement with extensive tissue binding in a peripheral compartment. Tissue binding may be more important than protein binding in this compartment, as the binding to human plasma proteins is only 21% [14]. The slow return into the central compartment may explain the relatively long period of time for which topotecan is detectable in plasma. The volume of distribution of the central compartment approximates that of total body water. This compartment provides the (physiological) pH, in which the hydrolysis reaction is continuously favored. This mechanism may determine mainly the elimination of the parent drug ($k_{10} \approx \lambda_f$), although renal or hepatic elimination can not be excluded.

The plasma concentration versus time plots of the topotecan metabolite could be described adequately using a one-compartment model (Eq. 3). After about 1.5 h the concentrations of the parent drug and metabolite decline in parallel, with identical slopes. Furthermore, since the time course of the metabolite is determined by the slowest step in the sequence of formation and elimination, which is the rate of formation ($\lambda_e > \lambda_f$), these data indicate that the metabolite has a shorter elimination half-life than the parent drug. Moreover, the ring-opened metabolite with a free and dissociated carboxylic function at physiological pH can be considered to be more highly polar and, hence, more readily and quickly eliminated from the body than the parent drug. The concentration of the metabolite in plasma might therefore be expected always to be lower than that of the parent drug. This, however, is not the case: the ratio between the plasma concentration of the parent and metabolite (Fig. 3) falls below 1 by 1 h after the start of infusion. A possible explanation might be the smaller volume of distribution of the metabolite, which is limited to the body water. Thus, the plasma concentrations do not reflect the total body amount of both forms. In fact, the central compartment contains about 17% of the total amount of topotecan present in the body, given by the ratio λ_2/k_{10}. The half-life for metabolite elimination, calculated from λ_e, is 29.0 (range 5.6–99.5) min, the half-life for metabolite formation, calculated from λ_f, is 123.2 (range 32–265) min.

The pharmacodynamic-pharmacokinetic plots could be described adequately by sigmoidal E_{max} models. Linear models were, generally, equally predictive, suggesting the modelling of the values of the pharmacokinetic parameters occurred in the linear part of the sigmoidal models. However, although similar results were obtained, on biological

grounds a sigmoidal model is more obvious and, therefore, preferable. The MTD (1.5 mg m^{-2} day^{-1}) administered in phase II studies would result in an average percentage decrease in ANC of 87%, as predicted by the sigmE_{max} model. The estimate for P_{50} (the dose associated with 50% decrease in ANC) is 0.6 mg m^{-2} day^{-1}. This is in accordance with the findings of others [9, 22] (i.e. 0.8 mg m^{-2} day^{-1}). These published pharmacodynamic studies also suggested that the relationship between the dose (mg m^{-2} day^{-1}) and total AUC and decrease (%) in ANC were the only ones [9, 22]. Other relationships with pharmacokinetic parameters could not be detected. This is in contrast to the present study, where plots of percentage decrease in ANC versus the dose, the AUC of topotecan, the AUC of its metabolite, and the total AUC were quite similar. The differences found in our study as against other reports may be explained in part by the sensitive and selective measurement of both forms. In other studies the HPLC method used was selective for only the lactone (closed) form of topotecan, whereas the ring-opened form was calculated as the total topotecan concentration (after sample acidification) minus the concentration of the lactone form [5, 9, 26, 33].

In conclusion, it is important that the pharmacokinetics of topotecan and its inactive metabolite are described, since this may help us to determine the optimal mode of drug administration and the optimal dosage, and extend our understanding of the differences found in pharmacodynamic outcome. Further research into the pharmacokinetic-pharmacodynamic relationships of topotecan is needed. For example, day-to-day variation within patients should be investigated, since the pharmacokinetics can vary very widely between days 1 and 4, as noted in two of our patients. With the aid of a limited-sampling model we have recently developed [29], which requires only one plasma concentration determination, we are now performing a pharmacokinetic study in which we determine AUC values on 5 consecutive days. With this information, the pharmacokinetic-dynamic relationships will be further explored. The encouraging results of phase I and II studies [17, 20, 24] legitimate this goal.

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