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Efficacy and toxicity profile of oral topotecan in a panel of human tumour xenografts

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Abstract

On the basis of their mechanism of action (cell killing during DNA replication) and the potential reversibility of the drug effects, protracted therapy with camptothecins is reported to provide optimal antitumour effects. Furthermore, oral administration may be a useful modality for optimisation of treatment. The aim of this study was to compare the therapeutic profile of topotecan given orally or intravenously in human tumours xenografted into athymic nude mice. The drug topotecan was given according to an intermittent (every fourth day, four times) or daily (qd×5/weekly×5−10 weeks; only orally) schedule. Tumour growth inhibition and persistence of drug effects were assessed and compared with untreated mice. In a panel of seven tumour xenografts, oral topotecan was at least as effective on three and significantly more effective on four tumours. Using the intermittent schedule, the maximum tolerated dose (MTD) was comparable for the two routes (15 mg/kg), but the toxicity profile suggested a better tolerability in terms of lethal effects after oral administration. The daily oral treatment of low drug doses allowed a higher cumulative dose to be delivered with improved antitumour efficacy (2/10 cured in a large cell lung cancer) and no evidence of toxicity. In spite of the low bioavailability of oral topotecan (23.5%), the persistent plasma levels of the drug suggest that the time of exposure to the drug is more critical than the plasma concentrations for antitumour efficacy. This interpretation is consistent with the increased efficacy of prolonged daily treatment with low-dose levels. The results may have implications for the future design of clinical studies.

Keywords: Administration routes; Camptothecins; Nude mice; Preclinical studies; Topotecan; Human tumour xenograft

1. Introduction

Camptothecins are antitumour drugs known to impair the topoisomerase I activity of mammalian cells by inducing single-strand DNA breaks; the primary DNA lesion may be converted to a lethal lesion (double-strand breaks) during DNA replication [1–3]. The S-phase specificity of the compounds [4] has suggested the importance of a prolonged exposure to achieve optimal antitumour effects. The hypothesis has been supported by preclinical and clinical studies. In particular, the camptothecin analogue topotecan (9-dimethylaminomethyl-10-hydroxy-(S)-camptothecin) has shown enhanced antitumour activity when administered for long periods of time [5,6].

The relevance of prolonged exposure for clinical development of camptothecins has recently been summarised [7]. A prolonged drug exposure may be more easily achieved by oral administration than by parenteral routes. Bioavailability and pharmacokinetics for oral topotecan have been investigated according to various treatment regimens [8–11]. A multicentre randomised phase III study of topotecan given intravenously (i.v.) versus orally in ovarian carcinoma patients has reported similar efficacy and lower myelotoxicity for oral administration than for the i.v. route [12]. However, the optimal regimen for topotecan treatment has yet to be determined. In preclinical systems, oral topotecan has been reported to be effective in the treatment of murine and human tumours [5,13] and pharmacokinetic data in mice for oral camptothecins are available [14]. However, a direct comparison of i.v. versus oral administration of topotecan has never been investigated in preclinical studies.

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The aim of the study was to compare the antitumour potential of topotecan, administered orally, on a panel of human tumours xenografted subcutaneously (s.c.) in athymic nude mice. The tumours were representative of several histotypes and responded differently to topotecan treatment. The comparison was carried out under various treatment conditions. The same regimen (schedule and dose) was investigated for both administration routes, and the daily administration was also investigated for the oral route. Pharmacokinetic analysis was also performed.

2. Materials and methods

2.1. Animals

Athymic Swiss nude mice, 9–12 weeks old (Charles River, Calco, Italy), were used throughout the study. Mice were maintained in laminar flow rooms, according to UKCCCR guidelines [15]. Experimental protocols were approved by the Ethics Committee for Animal Experimentation of the Istituto Nazionale Tumori, Milan.

2.2. Antitumour activity study

All tumour lines originated from subcutaneous (s.c.) injection of $5–10\times10^6$ cells from *in vitro* cultures (except COCF cells, which originated directly from a patient biopsy specimen) and were established in our laboratory according to previously described technical procedures [16].

Topotecan, kindly supplied by Smith-Kline Beecham Pharmaceuticals, was dissolved in sterile, distilled water and delivered in a volume of 10 ml/kg of body weight. The drug was administered by gavage or i.v. using the same schedule, i.e. every fourth day, four times (q4d ×4), which had been reported as effective in previous studies with parenteral delivery routes [5,17]. Many dose levels were investigated and the maximum tolerated dose (MTD) was considered the one inducing one death in the group of treated mice or a body weight loss up to 15%. A daily schedule using very low topotecan doses was also investigated for the oral route.

For the evaluation of antitumour activity, each control or drug-treated group included 4–6 mice bearing bilateral s.c. tumours which were implanted as tumour fragments by trocar. Tumour growth was monitored, and tumour volume (TV) was calculated by measuring tumour diameters with a Vernier caliper and using the formula: $TV = d^2\times D/2$, where d and D are the shortest and the longest diameter, respectively. Drug treatments started when tumours were visible and not measurable (< 100 mm^3). Tumour take in control mice was 100% and drug efficacy was evaluated as: (1) the percentage of TV inhibition in the treated versus control mice (TVI%), calculated by the formula: $100\text{-}(T/C\times100)$, where T is the mean TV of the treated and C that of the control mice, assessed 5–10 days after the last drug injection; (2) the $\log_{10}$ cell kill (LCK) achieved by the drug treatment, according to the formula $(T\text{-}C)/DT\times3.32$, where T is the mean time (days) required for the treated tumours and C for the control tumours to reach an established volume and DT is the mean doubling time of the control tumours. A tumour was considered sensitive when the drug treatment achieved a $TVI\% \geqslant 80$ and/or an $LCK \geqslant 1$.

Body weight loss and lethal toxicity were taken into account to evaluate the toxic effects induced by drug treatment. Animals were weighed twice a week and changes in mean body weight were calculated at each time as a percentage (%) compared with mean body weight at the beginning of treatment. The highest body weight loss % induced by treatments is reported in the Tables. In control mice, no body weight loss was recorded. Deaths occurring in treated mice were considered due to toxic effects. No control mouse died during the experimental time frame.

The Student's *t*-test (two tailed) was used for the statistical comparison of tumour volumes in mice treated with topotecan orally versus i.v.

2.3. Pharmacokinetics in mice

Healthy female athymic Swiss nude mice were treated i.v. or orally with 15 mg/kg of topotecan. At established times (5′, 15′, 30′, 1 h, 2 h, 4 h, 8 h, 24 h) 3 mice/group were bled from the retro-orbital sinus under light anaesthesia, and then sacrificed by cervical dislocation. Blood was centrifuged and plasma was stored at −20°C until high performance liquid chromatography (HPLC) analysis.

2.3.1. HPLC analysis

Mouse plasma was assayed for topotecan as the total lactone by using an HPLC fluorimetric method. For the determination of plasma levels, 100 μl of plasma were deproteinised with 150 μl of methanol. After vortexing and centrifugation (15 000 rpm, 10 min, 4°C) 50 μl of supernatant were injected onto the HPLC column (Phenomenex Luna C_{18} analytical column, 5 μm particle size, 4.5×15 mm, Torrance, CA, USA). The mobile phase was composed of 0.1 M acetic acid solution (adjusted to pH 3.5 with trimethylamine), and acetonitrile (80:20 v/v). The operating temperature was room temperature and the flow rate was 1 ml/min. The HPLC system consisted of a Model PU-980 pump (Jasco International Co. Ltd, Tokyo, Japan), a Model FP-920 fluorescence detector (Jasco International Co. Ltd), and a Model AS-950 autosampler (Jasco International Co. Ltd). The column eluate was monitored by fluorescence

with the excitation wavelength set at 370 nm and the emission wavelength set at 510 nm, gain 1000. Chromatography data processing was performed with Borwin Version 1.2.6 software (Jasco International Co. Ltd) working on a Pentium PC. Calibration curves were calculated by least square linear regression analysis with weight factor 1/y. Using 50 μl of plasma the lower limit of quantification for topotecan was 5 ng/ml. Linear response in the analytical peak area was observed for topotecan concentrations ranging from 5 to 100 ng/ml. The deviation from the nominal concentrations for all tested was equal to or less than 7%. Within run and between run precisions were less than 1.4% and average accuracy were between 1.7 and 5.3%.

2.3.2. Pharmacokinetic analysis

The pharmacokinetic parameters were obtained using a model-independent approach. For mean values of topotecan plasma concentration (three per time point), the maximum drug concentration (Cmax) and time to maximum drug concentration (Tmax) were generated directly from the experimental data. The area under the plasma concentration–time curve ($AUC_{0\text{-inf}}$) was estimated by the linear-logarithmic up to the last measured concentration time point ($AUC_{0\text{-t}}$) with extrapolation to infinity using the terminal rate constant k. On the mean plasma concentration versus time profile the data which represented the terminal part of the profile were visually selected and k was estimated by linear regression of the logarithm of the mean concentrations on sampling time. The apparent terminal half-life ($t_{1/2}$) was calculated as 0.693/k. The absolute bioavailability (F) was calculated as the ratio of the $AUC_{0\text{-inf}}$ after oral and i.v. administration. The pharmacokinetic analysis was carried out using WinNonlin Professional Edition, Version 1.5 software (Scientific Consulting Inc., USA).

3. Results

3.1. Antitumour activity studies

Using a panel of models characterised by varying responsiveness and different doubling times, the antitumour activity and the toxic effects (in tumour-bearing mice) induced by oral and i.v. topotecan delivery were studied according to the same schedule (q4d×4) (Tables 1 and 2). Against a prostate (JCA-1) and a large cell lung carcinoma (NCI-H460), two tumours highly responsive to the drug, a clear dose-dependent antitumour effect was observed either by oral or i.v. topotecan. The dose level of 15 mg/kg was the MTD by both routes, and oral topotecan was significantly more effective ($P < 0.05$) than i.v. topotecan against the two tumours (Table 1). At the MTD level, delivered according to the two routes, oral topotecan was significantly more effective than i.v. topotecan against the POVD lung tumour, achieving a TVI of 98% versus 87% ($P < 0.01$) and an LCK of 2.5 versus 1.1. Again, topotecan was more effective when given orally than i.v. against the U87 glioblastoma ($P < 0.05$ on TVI and LCK of 1.4 and 0.8, respectively). No appreciable differences in the antitumour effects were found when topotecan delivered by either route was compared in the other tumour lines investigated, i.e. the COCF colon, the SKOV-3 ovarian and the A549 lung carcinomas. Doubling times of the tumour lines investigated ranged between 4.2 (NCI-H460) and 8.6 (A549) days. The A549

Table 1
Antitumour activity of topotecan (q4d×4) on human tumour xenografts

Tumour type	Doubling time (days±S.D.)	Route	Dose[a] (mg/kg)	TVI%[b]	LCK[c]	Body weight loss %	Lethal toxicity[d]
NCI-H460	4.2±1.1	Oral	5	80	1.3		0/4
(Large cell lung)			9	91	1.7		0/4
			15	98*	2.1	8	0/4
			18	n.d.	n.d.	n.d.	2/4
		i.v.	10	65	0.5	6	0/4
			15	93	2.0	10	1/4
JCA-1	4.4±1.4	Oral	5	42	0.5	1	0/4
(Prostate)			9	66	0.6	6	0/5
			15	95*	1.5	15	0/5
			18	n.d.	n.d.	n.d.	2/4
		i.v.	12	74	0.9		0/5
			15	85	1.0	7	1/4

n.d., not determined; i.v. intravenous; q, every; d, day. *$P < 0.05$ versus the group treated with 15 mg/kg i.v. Student's *t*-test was used.

[a] Treatment started when tumours were visible and not measurable (< 100 mm^3).

[b] Tumour volume inhibition percentage in treated versus control mice, assessed 5–10 days after the last drug injection.

[c] Log_{10} cell kill.

[d] Number of toxic deaths/total number of treated mice.

Table 2
Antitumour activity of topotecan (15 mg/kg, q4d×4) on human tumour xenografts, using different routes of administration

Tumour type	Doubling time (days±S.D.)	Route[a]	TVI%[b]	LCK[c]	Body weight loss %	Lethal toxicity[d]
POVD	5.0±0.8	Oral	98†	2.5	13	0/6
(Small cell lung)		i.v.	87	1.1	1	0/5
U87	4.8±1.2	Oral	85*	1.4	7	0/6
(Glioblastoma)		i.v.	69	0.8	7	0/6
COCF	6.4±2.1	Oral	91	1.1	15	0/4
(Colon)		i.v.	91	1.2	1	0/5
SKOV-3	6.1±0.9	Oral	56	0.8	5	0/5
(Ovarian)		i.v.	54	0.6	6	1/4
A549	8.6±1.9	Oral	60	0.5	15	1/6
(Non small cell lung)		i.v.	42	0.5	8	1/6

i.v., intravenous; q, every; d, day. *$P<0.05$; †$P<0.01$ versus the i.v. treated group. Student's t-test was used.
[a] Treatment started when tumours were visible and not measurable (< 100 mm^3).
[b] Tumour volume inhibition percentage in treated versus control mice, assessed 5–10 days after the last drug injection.
[c] Log_{10} cell kill.
[d] Number of toxic deaths/total number of treated mice.

was the slowest tumour in the panel and the least responsive to topotecan by either route, using the intermittent treatment schedule.

Using the NCI-H460 lung cancer model the activity of oral topotecan was also studied by a daily schedule (qd×5/week) for 10 weeks. At the dose of 1.2 mg/kg/day, a TVI% of 87% and a LCK of 2.4 were achieved. Moreover, a higher dose level (2 mg/kg) completely inhibited tumour growth in 40% of mice and cured two out of ten tumours (at day 80) (Table 3). For regrowing tumours a LCK value of 6.0 was achieved. No body weight loss or toxic death occurred in mice treated with a cumulative dose of 100 mg/kg by the daily schedule (Fig. 1). The daily treatment was investigated also in the slowly growing A549 non-small cell lung tumours and a higher daily dose was tested (4 mg/kg). A clear therapeutic advantage was achieved in the group treated with topotecan according to the daily (qd×5/week for 5 weeks) compared with the intermittent (q4d×4) schedule (Fig. 2). In the former group, mice lost weight during treatment (maximum body weight loss 15%) but they recovered and no mouse died from toxicity. A cumulative dose up to 100 mg/kg could be safely delivered (Fig. 2).

Table 3
Complete responses to topotecan given orally against the NCI-H460 human large cell lung cancer xenograft

Schedule	Dose (mg/kg)		Complete response[a]	
	Single	Total	Day 30[b]	Day 80[b]
q4d×4	15	60	0/8	0/8
qd×5/w×10w	1.2	60	0/8	0/8
	2	100	4/10	2/10

q, every; d, day; w, week.
[a] Complete response: total disappearance of tumour.
[b] Calculated from the day of tumour xenograft (day 0).

Using the intermittent schedule, the reduction in body weight induced by topotecan was somewhat greater after the oral than after the i.v. treatment (Table 2). Table 4 summarises the toxicity results of all experiments reported in Table 2 together with those of other

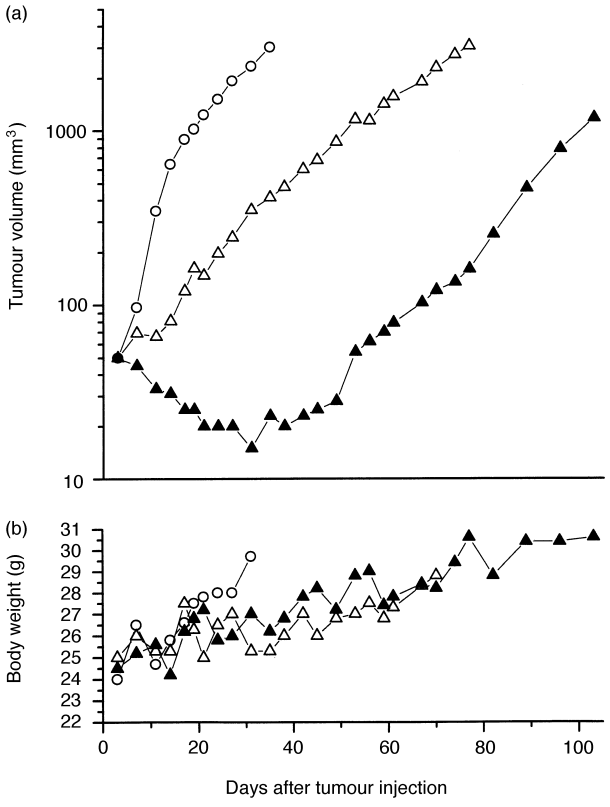

Fig. 1. (a) Growth curves of the NCI-H460 tumours treated with oral topotecan 1.2 mg/kg (△); 2 mg/kg (▲); or untreated (○). The drug was delivered daily 5 days/week for 10 weeks. Mean of 8–10 tumours/group is reported. (b) Body weight of mice bearing the NCI-H460 tumour. Same symbols as in (a). Mean of 4–5 mice/group is reported.

experiments not included in this study. Overall, 24 groups of tumour-bearing mice, for a total of 123 animals, were treated orally with topotecan (15 mg/kg), and in 6 of them a mean body weight loss $\geqslant 15\%$ (value considered relevant for drug toxicity) was achieved. However, only 2 mice died from toxicity. In contrast, in the 11 groups of mice treated i.v. with the same dose, mean body weight loss $\geqslant 15\%$ was never observed but lethal toxicity occurred in 5 out of the 55 treated mice. Therefore, using the q4d×4 schedule, in spite of a comparable MTD for the two tested routes, the pattern of toxicity was appreciably different.

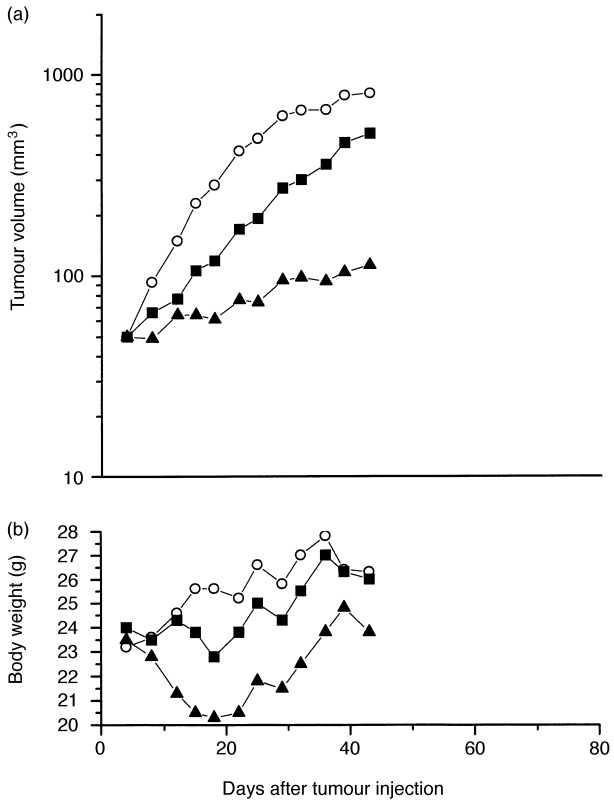

Fig. 2. (a) Growth curves of the A549 tumours treated with oral topotecan, 15 mg/kg, delivered q4d×4 (■); 4 mg/kg delivered daily ×5d/w×5w (▲); or untreated (○). Mean of 10 tumours/group is reported. (b) Body weight of mice bearing the A549 tumour. Same symbols as in (a). Mean of 5 mice/group is reported.

Table 4
Toxicity of topotecan (15 mg/kg, q4d×4) in tumour-bearing mice

Route	Experimental groups			Lethal toxicity[a]
	Total	With body weight loss		
		<15%	⩾15%	
Oral	24	18	6	2/123 (2%)
i.v.	11	11	0	5/55 (9%)

i.v., intravenous; q, every; d, day.
[a] Number of toxic deaths/total number of treated mice.

3.2. Pharmacokinetic analysis

The pharmacokinetic analysis of topotecan, 15 mg/kg, was performed after oral or i.v. delivery of the drug to healthy athymic mice. Fig. 3 shows the time-course of plasma levels of topotecan. Pharmacokinetic parameters for each route are reported in Table 5. After oral administration, topotecan was rapidly absorbed and reached the Cmax 0.5 h after treatment. The apparent $t_{1/2}$ was longer after oral than i.v. treatment. The absolute bioavailability for topotecan was approximately 24%.

4. Discussion

Camptothecins represent an important class of antitumour drugs endowed with the ability of targeting the

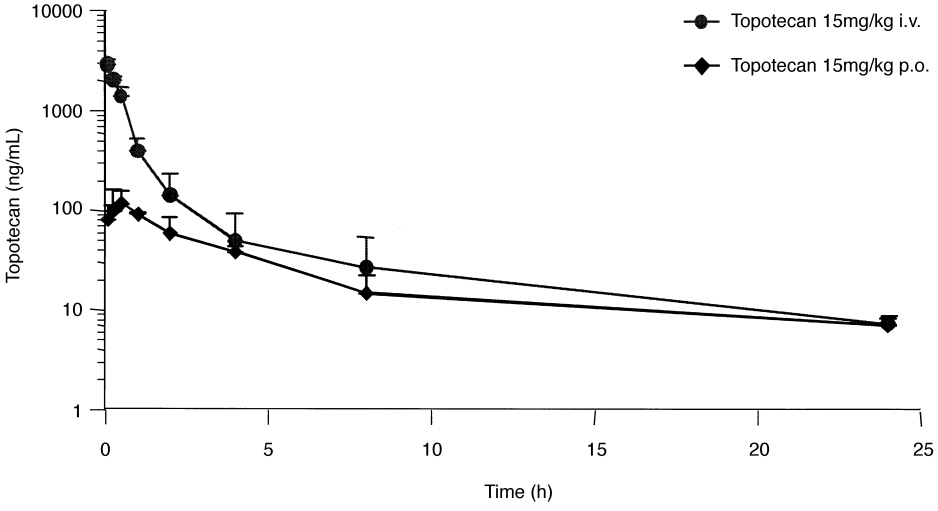

Fig. 3. Plasma concentration–time curves of topotecan (15 mg/kg) as the total of the lactone form after oral (◆) or i.v. (●) administration in nude mice. Mean (±S.D.) of 3 mice/time is reported.

Table 5
Pharmacokinetic parameters of topotecan after oral and i.v. administration (15 mg/kg)

Route	Tmax[a] (h)	Cmax[b] (ng/ml)	$AUC_{0\text{-}inf}$[c] (ng*h/ml)	$AUC_{0\text{-}t}$[d] (ng*h/ml)	k[e] (1/h)	$t_{1/2}$[f] (h)	F[g] (%)
Intravenous	0.08	2952.68	2454.14	2474.64	0.36	1.95	
Oral	0.5	118.91	552.59	580.91	0.25	2.77	23.5

[a] Time of maximal plasma concentration.
[b] Maximal plasma concentration.
[c] Area under the concentration–time curve from 0 to infinity.
[d] Area under the concentration–time curve from 0 to the last time point.
[e] Terminal rate constant.
[f] Apparent terminal half-life.
[g] Absolute bioavailability.

nuclear enzyme topoisomerase I [18]. Currently, camptothecin and four analogues are undergoing clinical evaluation [19]. One of them, topotecan, was developed as a water-soluble camptothecin and is used clinically for advanced ovarian and lung carcinoma [20,21]. The optimal therapeutic schedule for i.v. delivery of topotecan is still under study. Myelosuppression represents the dose-limiting toxicity in all studies [19].

The present study in preclinical human tumour systems documents that when delivered orally, topotecan showed a similar or greater antitumour activity than after i.v. treatment in all the tumour xenografts investigated. Using an intermittent (q4d×4) schedule, a significant improvement of activity, over i.v. administration, either in terms of the level of tumour inhibition or persistence of inhibition (as indicated by LCK values) was achieved in four out of the seven tumours (i.e. the U87 glioblastoma, the JCA-1 prostate carcinoma, the POVD small cell lung cancer and the NCI-H460 large cell lung cancer). All the four tumours were highly responsive to topotecan. In the SKOV-3 ovarian carcinoma and the A549 non-small cell lung cancer, both resistant to the drug, a comparable efficacy was observed. A tentative explanation of this pattern of tumour response could be that the improvement of drug efficacy following oral administration is related to intrinsic tumour responsiveness.

Our pharmacokinetic studies in mice showed a 23.5% bioavailability of topotecan after a single treatment of 15 mg/kg. However, in spite of the low bioavailability, orally given drug was present and detectable in plasma up to 24 h and its apparent $t_{1/2}$ was someway longer (2.77 h compared with 1.95 h) than that of the drug given i.v. The results indicate that drug persistence in plasma is a more critical determinant of tumour response than peak level, and support the importance of a prolonged tumour exposure to the drug for the antitumour efficacy of topotecan. Indeed, in spite of a low AUC value using the same therapeutic doses, the antitumour activity by the oral administration was fully maintained in the panel of human tumour xenografts investigated, and increased in the most responsive tumours. The high peak plasma level achieved after the i.v. delivery might be responsible for the incidence of lethal toxicity (possibly due to myelotoxicity) which was observed after i.v. and not after oral treatment.

The observation that the plasma persistence of the drug appeared to be more relevant for antitumour activity, suggested possible advantages of daily low doses of oral topotecan. When the cumulative dose of 60 mg/kg (the same used in the experiments with the q4d×4 schedule, 15 mg/kg) was fractionated by a prolonged (10 weeks) daily treatment (1.2 mg/kg/injection, qd×5/week), a comparable activity (LCK values: 2.1 and 2.4, respectively) was achieved in the NCI-H460 lung tumour. In addition, this schedule allowed the delivery of a higher total dose (2 mg/kg/injection, qd×5/week/×10 weeks, for a total of 100 mg/kg) without evidence of toxicity, but with a markedly increased antitumour efficacy (2/10 cured tumours) and a LCK value of 6. The therapeutic advantage of prolonged daily treatments of oral topotecan was more evident in the A549 lung tumour. Such a tumour line, characterised by a low proliferating rate (doubling time, 8.6±1.9 days), only partially responded to the intermittent schedule (60% TVI), but was highly inhibited by the daily treatment (85% TVI, Fig. 2). Again, the total dose of 100 mg/kg was well tolerated, even though delivered in a shorter time (5 versus 10 weeks).

On the basis of these results in clinical practice a therapeutic advantage may be expected by a daily delivery of oral topotecan. It is obvious that the oral route is more suitable than the parenteral one for daily schedules [22]. Oral bioavailability around 30% has been reported for topotecan in humans [8–11] and a large phase III study has documented a comparable efficacy of oral and i.v. topotecan in advanced ovarian cancer patients [12]. Our results suggest that the possible advantage of oral administration depends on the intrinsic tumour sensitivity, and it is conceivable that the lack of therapeutic benefit in patients with advanced ovarian carcinoma reflects the resistance of pretreated tumours.

When the drug was delivered by an intermittent schedule the MTD was unchanged after oral or i.v. treatment. The finding is consistent with the pharmacokinetic behaviour of the drug. However, the oral treatment was

better tolerated in terms of lethal toxicity than the parenteral one. In contrast, the oral treatment caused a more marked effect on mice body weight. Indeed, even though diarrhoea was not observed in mice, body weight loss may be considered suggestive of gastrointestinal toxicity which impairs food intake. Both the toxicities, haematological and gastrointestinal, are dose-limiting for the clinical use of camptothecins, but the latter is more manageable [19]. Further studies are required to better document the different pattern of toxicity of topotecan administered according to the two routes.

In conclusion, the oral delivery of topotecan was found to be a very effective route of treatment in human tumour xenografts, with the prolonged daily schedule achieving the best therapeutic index of the drug. In spite of a limited drug absorption following oral administration (probably related to the presence of P-glycoprotein in the intestinal mucosa), the low bioavailability was sufficient to ensure effective concentrations. Moreover, the toxicity pattern suggested a different profile of toxicity, in keeping with the results of a recent phase III study comparing oral and i.v. topotecan [12].

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