

Schedule-dependent Activity of Topotecan in OVCAR-3 Ovarian Carcinoma Xenograft: Pharmacokinetic and Pharmacodynamic Evaluation

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ABSTRACT

Topotecan is a topoisomerase (Topo) I inhibitor used in ovarian carcinoma chemotherapy. Topo I inhibitors are thought to be more cytotoxic using protracted schedules of administration. We tested this hypothesis on a preclinical model: human ovarian carcinoma OVCAR-3 implanted *i.p.* Nude mice were treated *i.p.* with a total dose of topotecan of 12.5 mg/kg delivered in 1, 5, 10, 20, 40, or 80 daily injections. The toxicity was maximal when the total dose was delivered within 5 and 10 days of treatment. However, the efficacy was the greatest (all of the mice cured) in the 20-day schedule using 0.625 mg/kg/day, hence, making this latter schedule the most efficient without any major toxicity. A pharmacokinetic study was conducted to identify parameters related to the efficacy and toxicity of topotecan in our model. The use of a population pharmacokinetic approach allowed us to define a therapeutic window: maintaining plasma concentrations above 0.2 μM for >10 h was necessary for an optimal antitumor effect and avoiding plasma concentrations >0.7 μM allowed a manageable toxicity. Finally, Topo I activity was monitored in ascites from animals treated with different topotecan administration schedules. The optimal schedule defined above allowed for sustained inhibition of Topo I activity associated with a greater antitumor activity. These *in vivo* data constitute a rationale for clinical studies testing this type of administration.

INTRODUCTION

Ovarian cancer is the most common fatal gynecological cancer in the developed countries. Although current chemotherapy regimens containing platinum derivatives can lead to re-

sponse rates of 60–80%, most patients ultimately relapse with platinum resistant disease (1–3). Topotecan, a water-soluble analogue of camptothecin, demonstrated its efficacy as a second line treatment and is registered in many countries as a single agent using five daily *i.v.* injections since 1996 (4–6). It inhibits specifically the activity of Topo² I by stabilizing the Topo I-DNA complex, resulting in a single strand DNA break, which may be converted into a lethal lesion during replication (7). Many different schedules of administration of this drug have been tested in preclinical studies (8–12) and in humans (13), but none of them has been defined as optimal, and clinical investigators are still assessing the optimal dose and schedule for this agent (14). Inhibitors of Topo I appear to be toxic only in S phase cells (15), and *in vitro* cytotoxicity is a function of exposure time above critical concentrations. Consequently, prolonged inhibition of Topo I should be considered as the important parameter in *in vivo* cytotoxicity (16). However, it remains to be determined whether exposure by continuous infusion or by prolonged daily administration offers the optimal differential between normal tissue damage and tumor cell kill.

Human ovarian carcinomas have been successfully xenografted *i.p.* into nude athymic mice (17, 18). Among these models, OVCAR-3 xenograft mimics the clinical situation, because tumor growth produces ascites, intra-abdominal carcinomatosis, and liver metastases.

The aim of this study was to compare the efficacy and the toxicity of different schedules of *i.p.* administration of topotecan using the same total dose in mice bearing OVCAR-3 ovarian xenografts. Pharmacokinetic/pharmacodynamic studies of topotecan were performed to determine the relationships between pharmacokinetics and both efficacy and toxicity and to define a therapeutic window. Finally, the impact of different schedules on Topo I activity was analyzed. These data could help in the definition of a better topotecan administration schedule for clinical use.

MATERIALS AND METHODS

Animals

Female Swiss athymic (6- to 8-week-old) mice, purchased from Iffa Credo (Saint Germain sur l'Arbresle, France), were housed in filter-capped cages kept in a sterile facility and maintained in accordance with the recommendations of the Federation of European Laboratory Animal Science Association. After a 2-week quarantine, they were used for both tumor maintenance and chemotherapy testing.

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² The abbreviations used are: Topo, topoisomerase; NB, nuclear buffer.

Tumor Model and Histology

The OVCAR-3 tumor model was originally established and described by Ortaldo *et al.* (19). A xenograft in nude mice was obtained after i.p. implantation of 4.5×10^7 cells of the NIH OVCAR-3 tumor cell line purchased from the American Type Culture Collection (Rockville, MD) and maintained *in vitro* in the laboratory. The i.p. xenograft was established, characterized, found stable after four passages and either maintained *in vivo* or kept frozen in liquid nitrogen. The i.p. xenograft was passaged as follows: the peritoneal cavity of mice with ascites was irrigated with sodium chloride solution (0.9%), and the washes were combined with the ascites. The cell suspension collected was rinsed twice in 10 ml of saline and centrifuged at $1000 \times g$ for 10 min. The pellet was resuspended and diluted one-third in saline solution. Each mouse received 1 ml of this cell suspension i.p., which represented $\sim 10\text{--}12 \times 10^6$ cells. The development of the abdominal carcinomatosis in mice was followed by the gain of weight, which was evident after 10–12 days. Mortality occurs under experimental conditions after ~ 60 days. Smears of the ascitic tumor at the first passage revealed groups and sheets of adenocarcinoma cells of variable size and shape containing pleomorphic nuclei. Subsequent passages (15) in nude mice produced no alterations in the tumor histology.

For experimental testing, the same protocol has been used: 1 ml of centrifuged ascites was diluted one-third with saline, and each mouse received 1 ml of cell suspension. The day of implantation was considered as day 0.

Drugs

Topotecan was kindly given by Smith Kline Beecham Pharmaceuticals. The drug was diluted in 0.9% sodium chloride solution and administered in a volume of 10 $\mu\text{l/g}$ of animal.

Chemotherapy Testing

Schedule Dependency of i.p. Administration of Topotecan. The total dose administered was fixed at 12.5 mg/kg, which corresponds to the maximal tolerated dose (MTD) determined by Houghton *et al.* (20) using a q4dx4 schedule.³ Maximal tolerated dose (MTD) this total dose was divided in 1, 5, 10, 20, 40, and 80 daily administrations. In the four last administration schedules, the i.p. injections were done 5 days/week for 2, 4, 8, or 16 consecutive weeks. The following different groups of mice were investigated:

- group 1 (1 day), administration of 12.5 mg/kg/day topotecan day 3;
- group 2 (5 days), administration of 2.5 mg/kg/day, days 3–7 (dx5);
- group 3 (10 days), administration of 1.25 mg/kg/day, days 3–7 and day 10–14 [(d \times 5) \times 2];
- group 4 (20 days), administration of 0.625 mg/kg/day, days 3–7, 10–14, 17–21, and 24–28 [(d \times 5) \times 4];³
- group 5 (40 days), administration of 0.312 mg/kg/day, days

3–7, 10–14, 17–21, 24–28, 31–35, 38–42, 45–49, and 52–56 [(d \times 5) \times 8]; and

group 6 (80 days), administration of 0.156 mg/kg/day, days 3–7, 10–14, 17–21, 24–28, 31–35, 38–42, 45–49, 52–56, 59–63, 66–70, 73–77, 80–84, 87–91, 94–98, 101–105, and 108–112 [(d \times 5) \times 16].

Intermittent Administration of Topotecan. The total dose of 12.5 mg/kg topotecan was used in two other administration schedules:

- group 7, administration of 1.25 mg/kg/day, days 3–7 and 17–21, (d \times 5)q2w;³ and
- group 8, administration of 0.625 mg/kg/day, days 3–7, 17–21, 24–28, and 38–42, [(d \times 5)q2w]2.

Schedule Dependency of i.v. Administration of Topotecan. The iv route was tested as following:

- group 1 (1 day), administration of 12.5 mg/kg/day topotecan, day 3;
- group 2 (5 days), administration of 2.5 mg/kg/day, days 3–7, [(d \times 5)];
- group 3 (10 days), administration of 1.25 mg/kg/day, days 3–7 and 10–14, [(d \times 5) \times 2];
- group 4 (20 days), administration of 0.625 mg/kg/day, days 3–7, 10–14, 17–21, and 24–28, [(d \times 5) \times 4].

Mice were monitored daily and weighed twice weekly until recovery of the initial weight. The efficacy of treatment was evaluated by animal survival (measured by the increase in lifespan, *i.e.*, the ratio between the median survival time of the treated group minus the median survival time of controls divided by median survival time of control group), the number of cured mice, and the presence or absence of ascites in the peritoneal cavity on the evaluation day.

Pharmacokinetic Study

Plasma pharmacokinetics of topotecan was determined in mice at four different dose levels: 12.5, 2.5, 1.25, and 0.625 mg/kg. Topotecan was given as a single i.p. dose. For the 2.5 and 0.625 mg/kg dose level, the pharmacokinetic study was repeated on day 5 to determine any potential accumulation of topotecan. Three animals/time point were sacrificed at 5, 15, and 30 min and 1, 2, 4, 6, and 24 h after injection. Blood was obtained by cardiac puncture. After immediate centrifugation, plasma was isolated and frozen at -20°C until analysis.

The total (*i.e.*, lactone plus hydroxy-acid forms) topotecan levels were determined using high-performance liquid chromatography as described previously (21). The limit of quantification was 1.2 nm of plasma. Three seeded plasmas with nominal values from 2.73 to 84.3 nm were used as quality controls to validate each high-performance liquid chromatography assay; the obtained concentrations should be within $\pm 10\%$ of the nominal values.

Pharmacokinetic Analysis

Plasma topotecan concentrations from all of the mice were analyzed simultaneously using the program NONMEM (version V, level 1.1; Ref. 22) with the first order estimation method and the PREDPP package (23) running on a PC computer. A proportional error model was used for both interanimal and residual variability. The choice of the structural pharmacokinetic model

³ (q4dx4): daily injection every 4 days for 4 cycles; (dx5)x2: daily injection for 5 consecutive days and 2 consecutive weeks; [(dx5)q2w]2: daily injection for 5 consecutive days repeated every 2 weeks for two cycles.

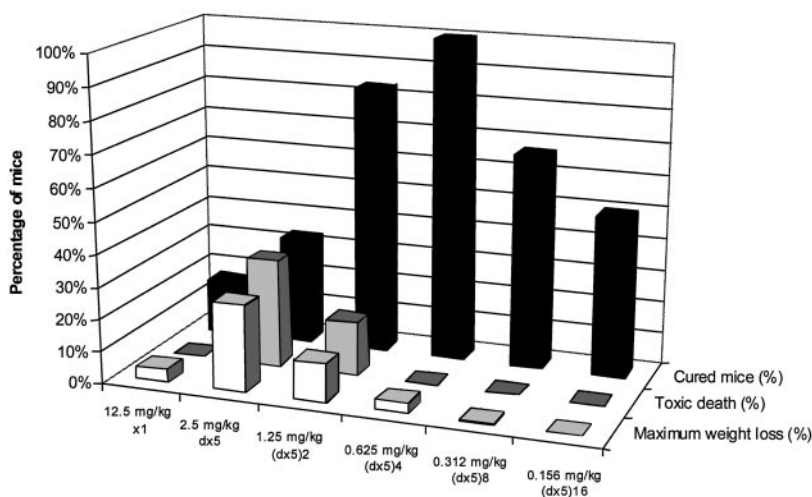


Fig. 1 Bell-shape profiles of both toxicity and activity of the different schedules of topotecan administered i.p. in mice. Toxicity was evaluated by the maximum weight loss of animals and the number of toxic deaths and antitumor activity by the number of cured mice. Topotecan was administered i.p. 1 day or 5 days/week repeated 1, 2, 4, 8, or 16 consecutive weeks.

was guided by graphical evaluation and by comparison of the objective functions corresponding to the different model tested (*i.e.*, one-, two-, and three-compartment model with first order absorption).

Pharmacodynamic Study

To compare the impact of different schedules of topotecan on the cytotoxicity both on normal and tumor tissues, mice were sacrificed, and ascites was recovered to determine Topo I activity. Cells pellets were frozen and stored at -80°C until analysis.

Only three different i.p. schedules, which showed a very different pattern in terms of both activity and toxicity, were considered for this experiment: group 1 consisting of a single topotecan administration, group 2 consisting of five topotecan administrations, and group 4 consisting of 20 administrations.

Preparation of Nuclear Extracts. Crude nuclear extracts were prepared as described previously (24). Briefly, cells were washed twice with cold NB [2 mM KH_2PO_4 , 5 mM MgCl_2 , 156 mM NaCl, 1 mM EDTA, 1 mM DTT (pH 6.5)], resuspended in 1 ml of NB containing 0.35% of Triton X-100 and 1 mM phenylmethylsulfonyl fluoride. The cell suspension was kept on ice for 10 min, washed twice with cold NB, and the nuclear protein was eluted for 1 h at 4°C with cold NB containing 0.35 M NaCl. After centrifugation at $18,000 \times g$ for 10 min at 4°C , the supernatant was collected and diluted in glycerol (50% final). The protein concentration was determined using the bicinchonidic acid method (25).

DNA Topo I Activity. The DNA Topo I activity was determined by measuring the relaxation of pKS plasmid (pBlue-script II; Stratagene, La Jolla, CA). The reaction mixture was comprised of 50 mM KCl, 5 mM MgCl_2 , 0.1 mM EDTA, 15 $\mu\text{g/ml}$ BSA, 10 mM Tris-HCl (pH 7.5), 0.5 mM DTT, 0.5 μg pKS, and different amounts of nuclear extract in a 20- μl final volume. After 10 min at 37°C , the reaction was stopped by addition of 1% SDS, 20 mM EDTA, 0.5 mg/ml proteinase K, and incubation was continued for an additional 30 min. After addition of 2.5- μl dye solution (10 mM Na_2HPO_4 , 0.3% bromphenol blue, and 16% Ficoll), samples were electrophoresed overnight

in a 1% agarose gel in Tris-borate EDTA migration buffer (90 mM Tris-borate 2 mM EDTA) at 30 V. Gel was stained with ethidium bromide and visualized on a UV transilluminator. Both relaxed and super coiled DNA were quantified using Image Quant software (Amersham Pharmacia Biotech, Piscataway, NJ). Topo activity was defined as the minimal amount of protein necessary to relax 50% of pKS in the assay conditions.

RESULTS

Schedule-dependent Antitumor activity. The percentage of cured mice according to the different schedules of administration is shown in Fig. 1. The activity of topotecan was optimal using 0.625 mg/kg/day ($d \times 5$)4 schedule and was minimal with the 12.5 mg/kg/day or 0.156 mg/kg/day ($d \times 5$)16 schedule. The toxicity profiles of the different schedules are shown in Fig. 1. The toxicity was maximal after five administrations of topotecan and minimal at the lowest daily doses.

Overall, both activity and toxicity presented a bell-shape profile according to the schedule of administration of topotecan, which was not superimposable (Fig. 1); this allows the definition of a very efficient and nontoxic schedule: 0.625 mg/kg/day, 5 days/week for 4 consecutive weeks.

Moreover, we tested two other efficient schedules [($d \times 5$)2 and ($d \times 5$)4] adding a week of rest between each weekly treatment (groups 7 and 8). A significant decrease in topotecan efficacy was observed (Table 1) with one cured mouse of six and three cured mice of six for the ($d \times 5$)q2w and [($d \times 5$)q2w]2 schedules, respectively.

Finally, the same schedule dependency of antitumor activity was confirmed using i.v. administration of topotecan. The results obtained in terms of maximum body weight loss and increase in life span of the different tested groups are presented in Table 2.

Pharmacokinetics of Topotecan in Mice. The profiles of topotecan plasma concentrations as a function of time are presented in Fig. 2. The two-compartment pharmacokinetic model with first order absorption (and no absorption lag time) was selected. This model fit the data without graphical evidence of dose-dependency phenomenon; the pharmacokinetics of to-

Table 1 Antitumor activity and toxicity of different schedules of topotecan administered i.p. in mice

Group of animals	Schedule of administration ^a	Toxic death (%)	Maximum weight loss (%)	Cured mice (%)
1	12.5 mg/kg × 1	0	4	16.67
2	2.5 mg/kg d × 5 ^b	33.3	27	33.33
3	1.25 mg/kg (d × 5) ^{2b}	16.67	12	83.33
4	0.625 mg/kg (d × 5) ^{4b}	0	3	100
5	0.312 mg/kg (d × 5) ^{8b}	0	0.15	50
6	0.156 mg/kg (d × 5) ^{16b}	0	0	33.33
7	1.25 mg/kg (d × 5)q2w ^c	0	18	16.67
8	0.625 mg/kg [(d × 5)q2w] ^{2c}	0	5	50

^a Topotecan treatment began 3 days after tumor implantation.

^b Topotecan was administered i.p. 5 days/week, for 1, 2, 4, 8, or 16 consecutive weeks.

^c Topotecan was administered i.p. 5 days/week every other week for a total of 4 or 8 weeks.

Table 2 Antitumor activity and toxicity of different schedules of topotecan administered intravenously in mice

Schedule of administration ^a	Life span (days)	Increased life span (%)	Maximum body weight loss (%)
Controls	59 ± 2.1		
12.5 mg/kg × 1	60.1 ± 14.6	0	1
2.5 mg/kg (d × 5) ^b	82.3 ± 3.4	40	7.3
1.25 mg/kg (d × 5) ^{2b}	77.8 ± 8	32	2.4
0.625 mg/kg (d × 5) ^{4b}	96 ± 6	63	0

^a Topotecan treatment began 3 days after tumor implantation.

^b Topotecan was administered i.v. 5 days/week, for 1, 2, or 4 consecutive weeks.

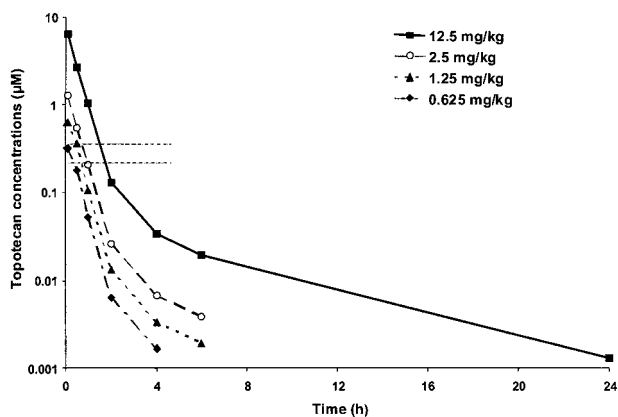


Fig. 2 Topotecan concentration time profiles after i.p. administration of different doses (12.5 mg/kg, 2.5 mg/kg, 1.25 mg/kg, and 0.625 mg/kg) in mice. Each point represents the mean of three independent samples.

potecan were linear between 0.625 and 12.5 mg/kg. In repetitive schedules, no accumulation in topotecan concentrations was observed. The mean pharmacokinetic parameters were: absorption rate constant = 13.4 h^{-1} ; central volume of distribution = 2.54 liter/kg; peripheral volume of distribution = 1.45 liter/kg; intercompartmental clearance = 0.425 liter/h/kg, and elimination clearance = 6.16 liter/h/kg. The elimination half-life was 2.5 h.

Pharmacokinetics-Pharmacodynamics Relationships.

The pharmacodynamic effects of topotecan (both efficacy and toxicity) appeared to be dependent of the administration schedule. Correlations between the time period during which plasma concentrations were maintained above a threshold concentration (during the overall treatment from 1 to 80 daily administrations) and the pharmacodynamic effects were tested. This was performed for successive topotecan concentrations differing by 0.1 μM whereby the time period during which plasma concentrations were maintained above the considered concentration was determined for each of the administration schedules by using the mean pharmacokinetic parameters. For each of the tested concentrations, time period and either efficacy or toxicity were plotted against each other and tested for correlation. The best correlation for efficacy (expressed by percentage of cured mice) corresponded to a plasma concentration maintained above 0.2 μM for >10 h. To avoid toxicity (expressed as the maximum of weight loss), plasma concentrations had to stay below 0.7 μM (Fig. 3).

Topo I Activity. The 1-, 5-, and 20-day schedules were investigated in terms of Topo I activity. Fig. 4 illustrates Topo I activity as a function of time according to different schedules of topotecan administration. After 1 day of treatment with 12.5 mg/kg topotecan, a very transient decrease in Topo I activity was observed, and the level returned to normal 3 days after treatment. Daily administration of 2.5 mg/kg for 5 days led to a 25% decrease in Topo I activity, but as soon as the treatment stopped the level returned to normal. Using the 20-day administration schedule, the same 25% decrease in Topo I activity was only achieved after 15 days of treatment. At day 19, the Topo I activity represented 30% of the basal level, which differed significantly from the nadir obtained after 5 days of treatment with 2.5 mg/kg ($P < 0.01$). After day 22, the determination of Topo I activity was not possible because of the absence of ascites in animals (all of the mice were cured with this schedule).

DISCUSSION

Camptothecins represent an important class of antitumor drugs targeting the nuclear enzyme Topo I and developed during the last 10 years. Among the four analogues of camptothecins, topotecan, a water-soluble camptothecin, is widely used for advanced ovarian cancer (4, 26). The optimal schedule for i.v.

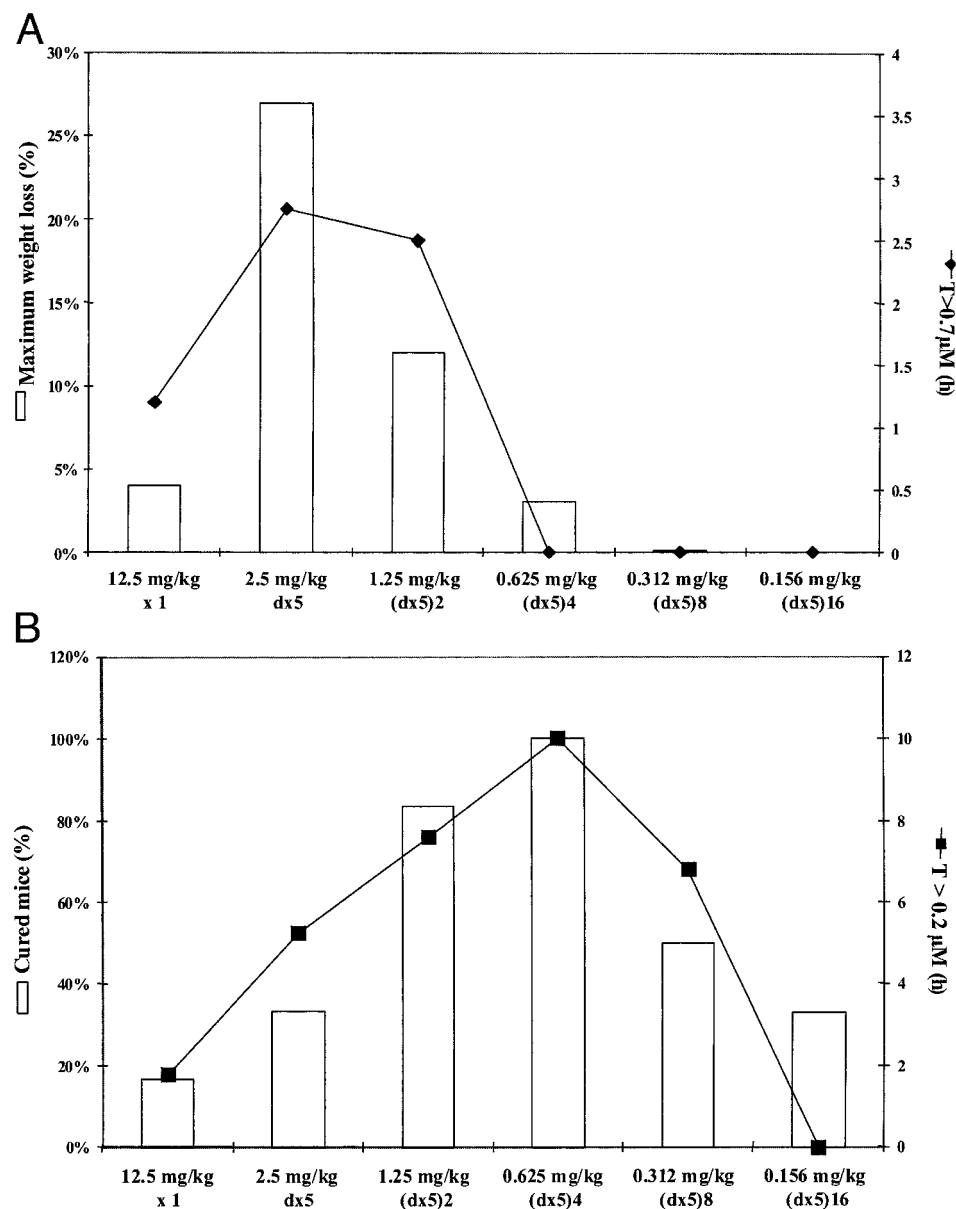


Fig. 3 Relationships between pharmacokinetics and pharmacodynamics of different schedules of topotecan administered i.p. in mice. A, relation between toxicity expressed as the maximum weight loss of animals and the time during which the plasma concentration was $>0.7 \mu\text{M}$ during the overall cycle of treatment. B, relation between antitumor activity expressed as the number of cured mice and the time during which the plasma concentration was $>0.2 \mu\text{M}$ during the overall cycle of treatment.

delivery is still under study. Myelosuppression represents the dose-limiting toxicity for most studies (14).

The present study in a preclinical human tumor system demonstrates that the fractionation of the same total dose from 1 to 80 administrations presents a bell-shape profile for antitumor activity; when topotecan was delivered using a protracted daily i.p. administration, a maximal activity was observed with a $(d \times 5)_4$ schedule. A similar pharmacodynamic profile was also observed for toxicity, but the maximum toxicity was noted after a 5-day treatment. There is a shift between the two bell-shape curves of efficacy and toxicity, and it was possible to define an optimal schedule of administration $[0.625 \text{ mg/kg/d } (d \times 5)$ for 4 consecutive weeks; Fig. 1]. These data confirmed that prolonged exposure schedules presented less toxicity and greater antitumor effect than high doses during short exposures

(8, 20). Furthermore, prolonged i.p. and p.o. administration of topotecan resulted in responses in xenografts that did not respond to a short term i.p. intermittent high-dose schedule, suggesting that prolonged administration could exert a greater cytotoxic effect on tumor cells (16).

Our study also investigated if a prolonged and continuous administration of topotecan was necessary to achieve a high antitumor effect. A dramatic loss in efficacy was noted in the schedules containing weekly rests (groups 7 and 8; Table 1). These data confirmed those observed by Houghton *et al.* (8, 27) with irinotecan. Reducing daily dose and increasing the period of administration appear to offer a therapeutic advantage in terms of both efficacy and toxicity.

Pharmacokinetic studies in mice confirmed the parameters obtained previously (11, 12, 20) with a low terminal half-life of

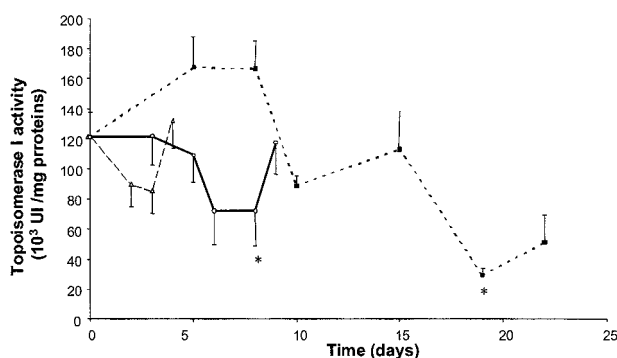


Fig. 4 Time profiles of Topo I activity in OVCAR-3 ascites cells after i.p. administration of different administration schedules of topotecan in mice. Each point represents the mean of three independent determinations. group 1: topotecan day 3, 12.5 mg/kg; group 2: topotecan 2.5 mg/kg/day (d × 5); and group 4: topotecan 0.625 mg/kg/day (d × 5)4. *, significant difference ($P < 0.01$).

topotecan not exceeding 2.5 h. The relationship between pharmacokinetics and efficacy allowed us to define a minimal efficient concentration in mice; the time during which plasma concentration was $>0.2 \mu\text{M}$ seemed to be critical for antitumor activity and maintaining this concentration for more than 10 h [(d × 5) × 4 schedule] exhibited the highest efficacy (100% of cured mice). In the schedules that did not maintain such a concentration during the same time period [dx1 and (d × 5) × 16 schedules], a lower efficacy was observed. By contrast, the high peak plasma level achieved after high doses of topotecan may have been responsible for the occurrence of lethal toxicity (possibly because of myelotoxicity; Ref. 12). A topotecan plasma level of $0.7 \mu\text{M}$ corresponds to a threshold; mice for which plasma levels were above that value for a long period of time exhibit maximal toxicity [d×5 and (d×5) × 2 schedules]. So in our animal model, a therapeutic window could be defined for topotecan; plasma concentrations had to be $>0.2 \mu\text{M}$ for >10 h without reaching $0.7 \mu\text{M}$ to obtain full activity without toxicity. This therapeutic window has been obtained after i.p. administration of the drug. Whereas a similar activity has been demonstrated by i.v. route, the threshold necessary for antitumor activity must be reconsidered for an i.v. administration.

The interaction of topotecan with its target Topo I as a pharmacodynamic end point has been evaluated in mice ascites on the course of treatment using 1, 5, or 20 [(d × 5) × 4] daily injections. The inhibition of Topo I differed between the three schedules of administration; when using a 1-day or a 5-day schedule, a very transient inhibition of Topo I was observed. With the (d × 5) × 4 schedule, a progressive inhibition of Topo I through the 3 first weeks of infusion was observed. After that time, animals did not present any ascites to allow additional investigation. The change in Topo I activity reached statistical significance only after 3 weeks of treatment ($P < 0.01$). However, this change did not preclude the antitumor activity of topotecan; the amount of Topo I-cleavable complexes was also a relatively good parameter to predict sensitivity to camptothecin derivatives, and parameters downstream from the cleavable complexes are also critical (7).

Many different administration schedules of topotecan have

been tested in Phase I and Phase II trials from a single injection to a 21-day continuous infusion (13). With a bolus infusion every 21 days, the MTD was 22.5 mg/m^2 , and the dose limiting toxicity was myelosuppression. At a dose level of 8.3 mg/m^2 , the toxicity was mild (only 1 of 7 patients developed a grade 4 neutropenia; Ref. 28). A similar total dose administered as a daily × 5 regimen ($7.5 \text{ mg/m}^2/\text{cycle}$) corresponds to the maximum tolerated dose (29, 30) with 38/61 cycles with grade 4 neutropenia (31). Finally, when a 21-day continuous infusion was tested, the MTD was $0.7 \text{ mg/m}^2/\text{day}$ i.e., $14.7 \text{ mg/m}^2/\text{cycle}$ (32). At the recommended dose, $0.4 \text{ mg/m}^2/\text{day}$ i.e., $8.4 \text{ mg/m}^2/\text{cycle}$, the toxicity was also mild; grade 4 neutropenia was only observed in 4% of 128 cycles (33). These clinical data confirmed the bell-shape curve observed for toxicity in mice; for a same total dose delivered, the 5-day schedule was limiting, and the 1-day or 21-day schedules have a manageable toxicity. Concerning the efficacy, with the 5-day schedule the overall response rate ranged between 9.5 and 22.6% in advanced epithelial ovarian cancer refractory to platinum regimen (4–6, 26). With a 21-day continuous infusion, Hochster *et al.* (33) demonstrated an overall response rate of 38% in another cohort of ovarian cancer treated previously with platinum-based chemotherapy. Overall, as in the mice model, protracted administration of topotecan enhanced efficacy and decreased toxicity. However, continuous infusion did not seem to be the optimal schedule, because this type of administration down-regulated the Topo I expression, which might constitute a mechanism of resistance to this drug. Then, repeated administration of topotecan should be favored. For this reason, p.o. administration should constitute a very attractive opportunity with relatively limited intrapatient variability of bioavailability (32–44%), low maximum plasma concentration, and the possibility of protracted schedule (34, 35). However, this schedule is associated with considerable gastrointestinal toxicity probably attributable to unabsorbed topotecan causing mucosal damage in the lower intestine.

Additional clinical trials should be designed to fully address the question of both the schedule of administration and the route of administration (i.v. bolus, p.o. administration, or continuous infusion). The improvement in toxicity management could constitute a chance for topotecan to be associated with other antitumor drugs.

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