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Evaluation of 9-dimethylaminomethyl-10-hydroxycamptothecin against xenografts derived from adult and childhood solid tumors

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Abstract. The topoisomerase I inhibitor 9-dimethylaminomethyl-10-hydroxycamptothecin (topotecan) was evaluated against a panel of xenografts comprising four lines of adult colon adenocarcinoma, three colon tumors derived from adolescents, six childhood rhabdomyosarcomas from previously untreated patients as well as sublines selected in vivo for resistance to vincristine and melphalan, and three lines of childhood osteogenic sarcoma. Efficacy was determined at maximal tolerated dose levels using intermittent i.p. administration [every 4 days for 4 doses (q4dx4)] or daily p.o. or i.p. administration 5 days per week for up to 20 courses. On a q4dx4 schedule, the maximum tolerated dose (MTD) was 12.5 mg/kg per administration, which caused marked weight loss and lethality in ≈5% of the tumor-bearing mice. This schedule caused significant growth inhibition (but no tumor regression) in advanced adult colon adenocarcinomas. The minimal treated/control (T/C) ratios were 0.49, 0.54, and 0.3 for three of the tumor lines and were achieved at 18–21 days after the initiation of treatment. In contrast, rhabdomyosarcomas were considerably more sensitive, with T/C ratios being <0.1 for three lines, whereas topotecan was less active against two other rhabdomyosarcoma xenografts (minimal T/C ratios, 0.17 and 0.14). As inhibitors of topoisomerase I have been demonstrated to have activity in the replication phase of the cell cycle (S-phase-specific), prolonged administration schedules were examined. Mice received topotecan 5 days per week for 3 weeks either by i.p. injection or by oral gavage (p.o.). In selected experiments, p.o. administration was continued for up to 20 weeks. Oral administration for 3 weeks (2 mg/kg per dose) resulted in complete regression of all six lines of rhabdomyosarcoma, with two lines demonstrating no regrowth during the period of observation (≥84 days). Similar results were obtained after i.p. administration, suggesting significant schedule dependency for these tumors. For colon tumors, the daily administration schedule (i.p. or p.o.) demonstrated some advantage over the intermittent schedule, resulting in partial regressions and significant inhibition of the growth of several colon adenocarcinoma lines. In rhabdomyosarcoma Rh12 and VRC_5 colon adenocarcinoma, both of which demonstrated intermediate sensitivity to topotecan, and in osteosarcoma OS33, protracted p.o. administration for 13–20 weeks (1.0–1.5 mg/kg per dose given daily ×5 days) caused complete regression without regrowth in Rh12 and OS33 tumors and partial regression of all VRC_5 tumors. No toxicity was observed using this schedule of administration. Topotecan demonstrated significant activity against all three osteosarcoma xenografts examined, with optimal schedules causing complete regression in two lines. Topotecan demonstrated similar activity against KB 3-1 and KB 8-5 multidrug-resistant cells in culture, and the Rh12/VCR an Rh18/VCR xenografts selected for vincristine (VCR) resistance in vivo were as sensitive as their parental lines. However, Rh28/L-PAM, selected for resistance to melphalan, was cross-resistant to topotecan. Plasma pharmacokinetics studies were carried out at the respective MTD for oral (2 mg/kg) or i.p. (1.75 mg/kg) administration. During oral administration the maximal plasma concentration (of the active lactone) was achieved at 0.25 h (C_{max} 41.7 ng/ml) and the $t_{1/2\alpha}$ and $t_{1/2\beta}$ values were 0.55 and 2.8 h, respectively. Administration i.p. resulted in peak plasma levels of 523 ng/ml, with $t_{1/2\alpha}$ and $t_{1/2\beta}$ elimination rates being 0.29 and 2.5 h, respectively. Although i.p. administration resulted in a 3-fold increase in AUC as compared with oral dosing, similar antitumor activity was observed against most xenograft lines. These results suggest that topotecan may have significant activity against several human cancers and that its efficacy may be schedule-dependent. Topotecan may have a particular role to play in the treatment of childhood solid tumors such as rhabdomyosarcoma and osteosarcoma.

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Introduction

Over the last decade, relatively few new effective agents have been introduced into the armamentarium for treatment of solid tumors. Indeed, development of aggressive therapy using ineffective agents has in many instances led to increased toxicity without resulting in benefit to the individual patient. Clearly, there is a need to identify new agents that have significant efficacy and to identify new targets against which effective inhibitors can be developed. However, the mechanism by which new agents are identified presents a formidable problem. Classic phase I/II trials against relapsed tumors in children may fail to identify, as active, agents that would have significant activity if used at diagnosis [14]. The problem is further compounded by the relatively few patients and, in many cases, the potentially curative therapy that compromise the process of evaluating new agents and introducing these into clinical management. In the treatment of refractory cancer such as colon adenocarcinoma, many new agents will be evaluated against relatively advanced disease, often in patients of advanced age in whom aggressive palliative therapy proves too toxic.

One, approach has been to develop preclinical models that may accurately identify new chemotherapeutic and biologic entities that have significant activitiy against specific malignancies. Our studies have used childhood rhabdomyosarcoma as a model of a chemosensitive histiotype [22, 23] and colon adenocarcinoma as a model of a chemorefractory tumor [17, 18]. More recently we have explored the potential for developing models of bone tumors [30]. In a coordinated effort, we have focused on the biology, genetics, and metabolic characteristics of these tumors so as to identify more accurately particular targets against which therapeutic agents can be directed and to develop in vivo models by heterografting tumor specimens into immune-compromised mice. The latter models have been used to identify both therapeutically active agents that affect known loci [22, 23] and agents with unknown mechanism(s) [25] that may prove valuable in identifying new targets for therapeutic intervention.

Rhabdomyosarcomas maintained as xenografts and cell lines derived from these appear to reflect accurately the genetic and biologic characteristics of the tumor of origin. Specifically, histologic integrity, cytogenetic abnormalities, and myogenic markers are retained in rhabdomyosarcoma heterografts [8, 13, 21]. It is noteworthy that chemosensitivity profiles of tumors directly established in vivo appear closely to parallel rhabdomyosarcomas in the clinic, suggesting that these models may be valuable for the identification of new agents that may have significant therapeutic utility in curative treatment of this tumor. In contrast to rhabdomyosarcomas, colon adenocarcinomas derived from adults or adolescents are quite refractory to almost all agents evaluated when they are grown under identical conditions in immune-deprived mice (reviewed in [26]). Preliminary studies on osteosarcoma xenografts indicate that these tumors maintain genetic characteristics of the disease such as deleted expression, or expression of an altered transcript, of Rb^1 and retention of multiple stem lines in the mouse host [30].

In the present study we evaluated 9-dimethylaminomethyl-10-hydroxycamptothecin (topotecan), a specific inhibitor of topoisomerase I [28]. This enzyme relaxes supercoiled DNA and appears to be important for semiconservative replication of double-helical DNA, transcription, recombination, and chromosomal decondensation [36]. The antitumor activity of 20(S)-camptothecin, a plant alkaloid isolated from *Camptotheca acuminata*, was studied in the early 1970's, [12]. Camptothecin was evaluated clinically in the United States as the sodium salt but was found to be ineffective in patients with advanced disseminated melanoma or gastrointestinal cancer [11, 32], although clinical trials in China using 10-hydroxycamptothecin demonstrated activity in the treatment of gastric cancer, head and neck tumors, and bladder carcinoma (reviewed in [28]). Unpredictable and severe toxicities included myelosuppression, vomiting, diarrhea, and hemorrhagic cystitis that resulted in the discontinuation of phase II trials of camptothecin in the United States. Recently, Giovanella et al. [10] reported the curative activity of 20(RS)-9-aminocamptothecin against early-stage colon adenocarcinoma xenografts following administration of the drug by subcutaneous implant. An extensive program to develop water-soluble inhibitors of topoisomerase I resulted in the selection of topotecan as a candidate for human trials [28]. This agent is currently undergoing phase II evaluation in adults and has recently entered phase I testing in the pediatric population.

Materials and methods

In vitro studies. Cell lines KB3-1 and $KBCh^R8$-5 were obtained from Dr. I. Pastan and maintained in antibiotic-free medium containing 10% fetal calf serum (Gibco). $KBCh^R8$-5 was grown in the presence of 10 ng colchicine/ml [1], and it overexpresses the multidrug-resistance (*mdr*)1 gene 16-fold relative to the parenteral KB3-1 clone [16]. For drug-sensitivity studies, cells were seeded at $2 \times 10^5/cm^2$ and exposed 24 h later to cytotoxic agents continuously for 72 h. Cell number was determined by counting nuclei after lysis of cells [25].

Immune deprivation of mice. Female CBA/CaJ mice (Jackson Lab, Bar Harbor, Me.) aged 4 weeks were immune-deprived by thymectomy and were subjected to whole-body irradiation (950 cGy) using a ^{137}Cs source 3 weeks thereafter. Mice received 3×10^6 nucleated bone marrow cells within 6–8 h of irradiation [25].

Tumor lines. Four of the six independently derived lines from previously untreated rhabdomyosarcoma (RMS) have been described elsewhere [8, 13, 21, 25]. Two additional lines, IRS-56 and IRS-68 (both embryonal), were established from tissues obtained through the Intergroup Rhabdomyosarcoma Study (designated IRS). The characteristics of each xenograft are summarized in Table 1. For chemotherapy studies, all tumors were used within 22 passages of their engraftment in mice. Each tumor grows routinely in over 90% of recipient mice, and all are human as determined by karyotype and species-specific isoenzyme patterns. The chemosensitivity of these lines has previously been reported for conventional agents in the therapy of RMS [22] and for melphalan (L-PAM) [23].

Sublines of Rh12, Rh18, and Rh28 selected in situ for resistance to vincristine (VCR); sublines Rh12/VCR-3 and Rh18/VCR-3) and L-PAM (Rh28/L-PAM) have been described elsewhere [15, 24]. The colon adenocarcinomas used (HC_1, GC_3, and VRC_5) were derived from adult patients and have been extensively characterized [19, 20, 25]. SJC_2 was established from a moderately differentiated primary colon lesion in a

Table 1. Characterization of xenografts

Code	Histology	Patient		Reference
		Sex (M/F)	Age (years)	
Colon adenocarcinomas (adult):				
HC_1	Moderately well differentiated	F	68	[19, 20]
GC_3	Poorly differentiated	M	61	[19, 20]
VRC_5	Poorly differentiated	M	72	[19, 20]
ELC_2	Poorly differentiated, signet-ring cell	F	83	[19, 20]
Colon adenocarcinomas (juvenile/young adult):				
SJC_2	Poor to moderate differentiation	F	14	Unpublished data
SJC_3A	Poor to moderate differentiation	M	26	Unpublished data
SJC_3B	Moderately well differentiated	M	26	Unpublished data
Childhood rhabdomyosarcoma:				
Rh12	Embryonal (site, buttock)	M	3	[21]
Rh18	Embryonal (site, perineum)	F	1	[13, 21]
Rh28	Alveolar (site, lymph node)	M	17	[13]
Rh30	Alveolar (site, bone marrow)	F	16	[8]
IRS56	Embryonal (site, buttock)	M	3	Unpublished data
IRS68	Embryonal (site, shoulder)	M	13	[25]
Childhood osteosarcoma:				
OS2	Large-cell/chondroid	F	14	[29]
OS9	Fibroblastic/osteoid	M	11	[29]
OS33	Osteoblastic undifferentiated	F	8	[30]

14-year-old girl. SJC_3A and JSC_3B tumors were independent primaries in a 26-year-old male. Osteosarcoma xenografts OS2, OS9, and OS33 have been described previously [30].

Growth-inhibition-studies. Therapy was started in mice bearing bilateral subcutaneous tumors when the lesions had reached a diameter of approximately 0.5–1 cm. Tumor response was determined at 7-day intervals using digital calipers (Maxcal) interfaced to an IBM PS/2 microcomputer. Two perpendicular diameters were used to compute volumes [25]. Growth delay was calculated from the difference in days required for treated tumors to grow to 4 times their pretreatment volume as compared with vehicle-treated controls. For each treatment group, six or seven tumor-bearing mice were used. Relative tumor volumes (RTV) were calculated using the formula $RTV = (V_x/V_o)$, where V_x is the volume on day x and V_o is the volume of the tumor at the initiation of treatment. Treated/control (T/C) ratios for tumor volumes were calculated from the RTV values after each measurement. The minimal T/C ratio i.e., the smallest T/C ratio) and the day on which it was achieved are presented. To equate responses in tumor lines that demonstrate different rates of growth, we normalized inhibition by expressing it as a function of tumor volume-doubling time. Grading of tumor responses is given in Table 2; the definition of $\geq 50\%$ regression required that each tumor within a given group at a certain time point after treatment demonstrate such a reduction in volume.

Formulation and administration. Topotecan was dissolved in 0.9% saline for i.p. administration and in water for oral gavage (0.1 and 0.05 ml/10 g body weight, respectively). Topotecan was generously provided by Dr. R. K. Johnson, Smith Kline Beecham.

Pharmacokinetics of topotecan in mice. Following a single dose of either oral (2 mg/kg) or i.p. (1.75 mg/kg) topotecan, blood samples were collected from mice (four animals per point) at 0.25, 0.5, 1, 1.5, 3, 4, 6, and 8 h. All samples were immediately centrifuged at 1250 *g* for 2 min on a tabletop centrifuge. Plasma proteins were precipitated by the addition of 200 µl plasma to 800 µl cold methanol (–30° C) followed by vigorous agitation on a vortex and recentrifugation at 1250 *g* for 2 min. The supernatant was decanted and stored at –70° C until analysis. Plasma concentrations of topotecan were measured by an isocratic high-performance liquid chromatographic (HPLC) method using fluorescence detection [2]. This method was determined to be precise (intraday and interday CV, 0.4%–3.7%) and accurate (mean prediction error, from 0.2% to –3.4%) at topotecan concentrations ranging from 1.5 to 400 ng/ml.

Pharmacokinetic analysis. A two-compartment model was fit to the topotecan plasma concentration-time data by the use of maximal likelihood estimation [6]. The parameters estimated included the volume of the central compartment (V_c), the elimination rate constant from the central compartment (K_{el}), the intercompartment rate constants (k_{cp} and k_{pc}), and, for oral dosing, the absorption rate constant (k_a).

Statistical analysis. The results of individual tumor-inhibition studies were analyzed by one-way analysis of variance using the number of days to reach 4 times the original tumor volume as the dependent variable. Only tumors from mice that survived the entire study were included in the analyses, and any tumor that did not reach 4 times its original volume was assigned a default value for the maximal duration of the study. To compare the efficacy of various courses of treatment, data were collapsed across studies within a tumor line. The percentages of tumors showing partial and/or complete regression and any regrowth were calculated for the individual tumor lines as described elsewhere [25].

Results

In vitro studies

Expression of P-glycoprotein has been detected in each of the tumor types under study [4, 5] and has been correlated with poor outcome [4, 5]. One criterion that we use to prioritize in vivo evaluation requires that a new agent be equally active against cells that exhibit a typical multidrug-resistant (MDR) phenotype conferred by the *mdr*1 gene product. As shown in Fig. 1, $KBCh^R8$-5 showed approximately 47-fold resistance to VCR as compared with the parental KB3-1 cell line; in contrast, there was very little cross-resistance to topotecan (approximately 1.8-fold).

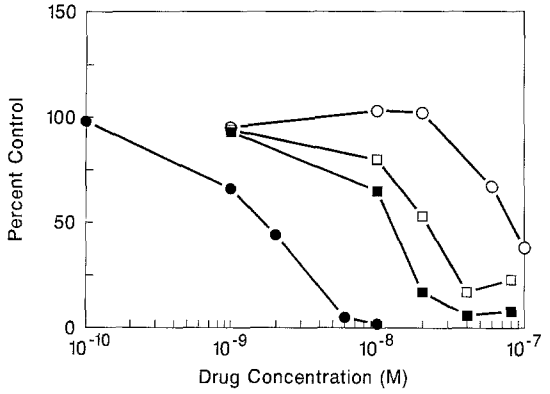

Fig. 1. Sensitivity of KB3-1 (*closed symbols*) and KBCh^{R}8-5 (*open symbols*) cells exposed for 72 h to VCR (●, ○) or topotecan (■, □). Each point represents the mean value for 3 determinations (SD, <5%)

In vivo studies

Colon adenocarcinomas. Initially, three lines derived from adult malignancies were evaluated using a q4dx4 schedule. The MTD was 12.5 mg/kg, which caused 5% lethality (4/84 mice). These tumors have been extensively studied and are intrinsically resistant to most therapeutic agents (reviewed in [18]). Tumor growth-inhibition data are presented in Table 2. Topotecan caused significant growth delay in VRC_5 tumors but did not induce any tumor regression of ≥50%. The activity of topotecan against these colon tumors was analyzed for drug-induced growth inhibition, minimal T/C volume ratios, and tumor regression.

Rhabdomyosarcomas. At the same doses and on the same schedule of administration (q4dx4), topotecan caused significant regression of advanced Rh12, Rh18, Rh28, and Rh30 xenografts. Rh28 tumors were highly sensitive to topotecan, and complete regression with subsequent regrowth was obtained using 12.5 mg/kg per dose. Data for all rhabdomyosarcomas are summarized in Table 2.

Effect of scheduling topotecan

It has previously been proposed that the exposure time rather than the concentration per se may be more important as a determinant of response for cell-cycle-specific agents (e.g., cytosine arabinoside, VP-16 [7]). A similar situation may pertain to inhibitors of topoisomerase I, which act

Table 2. Responsiveness of xenografts to high-dose intermittent-schedule topotecan

Tumor	Dose[a]	Time to 4X ± SD[b] (days)	Growth delay (days)	T/C[c] (min)	% Response[d]			Response ranking[e]
					PR	CR	C	
Colon adenocarcinomas:								
HC_1	0	46.8±21	–	–	–	–	–	
	12.5	54.7± 6.5	7.9	0.49 (21)	0	0	0	–
GC_3	0	31.0±19.6	–	–	–	–	–	
	12.5	42.3±17.9	11.3	0.54 (21)	0	0	0	–
VRC_5	0	14.6± 6.0	–	–	–	–	–	
	10	27.4± 8.3	12.8*	0.50 (18)	0	0	0	+
	12.5	75.5±16.5	60.9*	0.30 (18)	0	0	0	+++
Rhabdomyosarcomas:								
Rh12	0	29.3± 6.6	–	–	–	–	–	
	10	50.8±13.5	21.5*	0.31 (27)	7	7	0	+
	12.5	61.0±14.5	31.7*	0.17 (31)	64	57	28	++
Rh18	0	13.8± 5.5	–	–	–	–	–	
	10	34.7±10.8	20.9*	0.15 (18)	49	0	0	+++
	12.5	75.5±16.5	61.7*	0.05 (18)	100	71	43	++++
Rh28	0	24.5± 7.5	–	–	–	–		
	12.5	65.1±16.5	40.6*	0 (18)	100	100	28	+++++
Rh30	0	16.1± 6.4	–	–	–	–	–	
	10	45.5± 6.4	29.4*	0.09 (21)	43	0	0	+++
	12.5	46.5± 3.7	30.4*	0.09 (21)	35	0	0	+++
IRS68	0	18.3± 9.8	–	–	–	–	–	
	12.5	31.2±11.1	12.9	0.34 (13)	18	0	0	+

[a] Dose: mg/kg q4d × 4 i. p.

[b] Time required for tumors to grow to 4 times the volume measured at the initiation of treatment

[c] Minimal T/C ratio; the day after the start of treatment is shown in parentheses

[d] Partial response (PR), (≥50% regression; complete response (CR) ≥90% regression; C, no regrowth of tumor during the period of observation (84 days)

[e] Tumor response criteria: –, no growth inhibition; +, ≥Td_2; ++, ≥2 × Td_2; +++, growth inhibition of ≥3 × Td_2; ++++, growth inhibition of >3 × Td_2 plus volume regression of ≥50%; +++++, complete regression with subsequent regrowth; ++++++, complete regression with no growth during the period of observation (≥84 days). Td_2, mean time required for tumor volume to double

* Significantly different from control values (P <0.05)

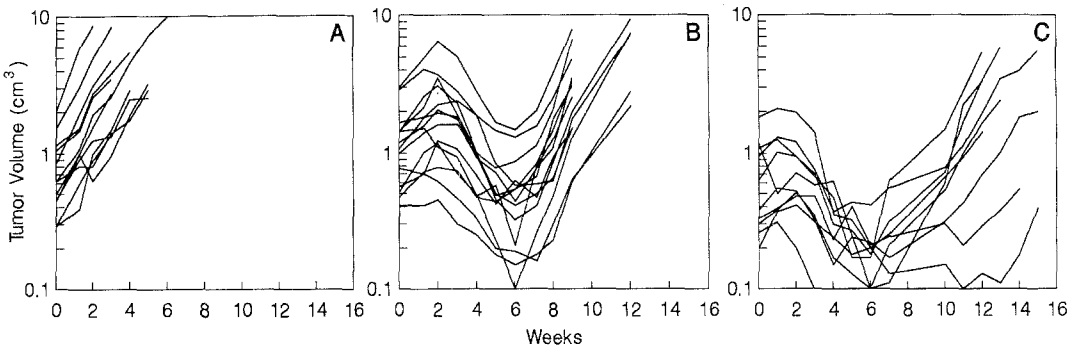

Fig. 2 A – C. Responses of VRC_5 colon adenocarcinoma xenografts to topotecan. Mice received vehicle (**A**) or topotecan at a dose of 1.75 (**B**) or 2 mg/kg (**C**) p. o. on 5 consecutive days each week for 3 weeks. The graphs demonstrate the growth patterns of individual tumors

predominantly or exclusively in the S phase of the cell cycle [3]. To examine this possibility, mice were treated by daily administration of topotecan 5 days per week for 3 consecutive weeks [(dx5) schedule]. As preliminary studies on murine syngeneic tumors have demonstrated the efficacy of topotecan given by oral gavage (R. K. Johnson, personal communication), this route of administration was chosen for initial evaluation. The results of studies using seven lines of colon adenocarcinomas are presented in Table 3. The maximal dose used was 2 mg/kg per administration, which caused no lethality and minimal weight loss. At the end of the therapy, mice weighed >97% of their pretreatment weight. Topotecan given p.o. at 2 mg/kg per dose also caused partial regression of some advanced HC_1, VRC_5, and SJC_3B xenografts. The growth curves generated for the most responsive colon adenocarcinoma line, VRC_5, are presented in Fig. 2. At the highest nontoxic dose evaluated, topotecan caused significant growth delay in HC_1, VRC_5, SJC_3A, and SJC_3B xenografts.

The responsiveness of rhabdomyosarcoma xenografts to daily oral administration of topotecan is presented in Table 4. Complete regression of all tumors was achieved in Rh28, Rh30, IRS56, and IRS68 xenografts together with significant growth inhibition. Rh12 and Rh18 xenografts were less responsive, although >70% of the tumors regressed partially at the highest doses used. Topotecan also caused partial regression of very advanced Rh18 xenografts (Table 4). It is noteworthy that there was little evidence of a dose-response relationship in these tumors.

The efficacy of parenteral (i.p.) administration of topotecan on a $(dx5)_3$ schedule was also examined. Parenteral administration was at least as efficacious as oral

Table 3. Responsiveness of colon adenocarcinoma xenografts to oral topotecan

Tumor	Dose[a]	Time to 4X ± SD (days)	Growth delay (days)	T/C (min)	% Response			Response ranking[b]
					PR	CR	C	
HC_1	0	24.9± 7.1	–	–	–	–	–	
	1.5	45.2± 7.5	20.3*	0.32 (28)	8	0	0	+(+)
	2.0	55.8± 8.4	30.9*	0.21 (28)	21	7	0	++
GC_3	0	17.6± 4.9	–	–	–	–	–	
	1.5	26.0± 5.2	8.4	0.38 (7)	0	0	0	+
	1.75	33.5±12.4	15.9	0.27 (7)	0	0	0	+
VRC_5	0	21.1± 5.7	–	–	–	–	–	
	1.5	69.1±13.6	48.0*	0.08 (42)	79	0	0	+++
	2.0	66.9±14.1	45.8*	0.06 (42)	80	20	10	+++
ELC_2	0	36.7±12.0	–	–	–	–	–	
	1.5	57.0±21.0	20.3	0.23 (49)	0	0	0	+
	1.75	56.4±22.5	19.4	0.25 (42)	7	0	0	+
SJC_2	0	19.2± 9.1	–	–	–	–	–	
	1.5	31.3±10.5	12.1	0.34 (21)	0	0	0	+
	1.75	46.4±22.5	27.2	0.60 (21)	0	0	0	++
SJC_3A	0	18.2± 4.3	–	–	–	–	–	
	1.5	34.2±11.6	16.0	0.37 (21)	7	0	0	+
	1.75	30.3± 8.0	12.1	0.49 (21)	0	0	0	+
	2.0	56.1±15.3	37.9*	0.25 (21)	0	0	0	+++
SJC_3B	0	26.1± 6.9	–	–	–	–	–	
	1.5	36.1± 5.3	10.0	0.49 (28)	0	0	0	–
	1.75	44.3± 7.8	22.2*	0.29 (28)	0	0	0	+
	2.0	62.5±10.9	37.4*	0.18 (28)	50	0	0	+++

[a] Dose: mg/kg (daily, x 5) for 3 courses p.o.; for definitions of other parameters, see Table 2

[b] The response shown in parentheses indicates the maximal variation between replicate experiments

* Significantly different from control values (P <0.05

Table 4. Responsiveness of rhabdomyosarcoma xenografts to oral topotecan

Tumor	Dose[a]	Time to 4X ± SD	Growth delay (days)	T/C (min)	% Response			Response ranking[b]
					PR	CR	C	
Rh12	0	20.2± 9.0	–	–	–	–	–	
	1.0	58.5± 6.5	41.9*	0.07 (28)	36	0	0	+++
	1.75	67.0±11.6	46.8*	0.07 (28)	72	36	27	+++(+)
	2.0	70.5± 9.2	50.4*	0.07 (42)	75	25	0	+++(+)
Rh18	0	14.8± 7.0	–	–	–	–	–	
	1.5	69.4±22.6	54.6*	0.02 (14)	92	83	50	+++(+)
	1.75	52.2±23	37.4*	0.03 (14)	71	50	28	+++(+)
Advanced Rh18	0	18.5± 6.9	–	–	–	–	–	
	2.0	43.5± 8.0	25.0*	0.09 (21)	57	0	0	+++
Rh28	0	18.7± 7.0	–	–	–	–	–	
	1.0	59.2±20.6	40.5*	0.06 (21)	85	64	35	+++(+)
	1.5	72.8±13.6	54.1*	0.0 (16)	100	100	50	+++++
	2.0	78.7± 9.2	60.0*	0.0 (16)	100	100	63	+++++
Rh30	0	19.8± 8.9	–	–	–	–	–	
	1.0	>84	>64*	0.0 (21)	100	100	100	++++++
	1.5	>84	>64*	0.0 (21)	100	100	17	+++++
	2.0	>84	>64*	0.0 (21)	100	100	40	+++++
IRS56	0	33.4±10.8	–	–	–	–	–	
	1.0	56.6± 9.9	23.2*	0.05 (42)	100	86	29	++++
	1.5	>84	>50*	0.02 (49)	100	85	85	++++
	2.0	>84	>50*	0.02 (49)	100	100	86	+++++
IRS68	0	13.5± 6.5	–	–	–	–	–	
	1.0	>84	>70*	0.02 (35)	100	86	29	++++
	1.75	>84	>70*	0.0 (35)	100	100	100	++++++

[a] Dose: mg/kg (daily × 5) for 3 courses p.o.; for definitions of other parameters, see Table 2

[b] Values shown in parentheses indicate maximal variations between replicate experiments

* Significantly different from control values ($P < 0.05$)

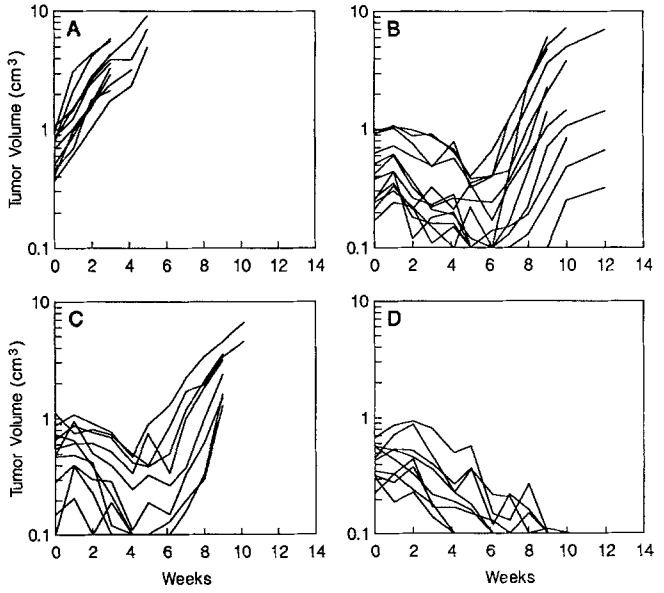

Fig. 3A–D. Responses of Rh12 rhabdomyosarcoma xenografts to topotecan. Mice received either topotecan on a $(dx5)_3$ p.o. schedule or vehicle. **A** Control. **B** 2 mg/kg per dose. **C** 1.75 mg/kg per dose. **D** 1.25 mg/kg per dose for 13 courses. The graphs show the growth curves generated for individual tumors

dosing, albeit clearly more toxic, and the toxicity resulting from the former was also less predictable. The administration of 2 mg/kg per dose i.p. was lethal in >15% of the mice, and 1.5–1.75 mg/kg per dose represented an approximate MTD (LD_{10}). The data shown in Table 5 summarize the tumor-growth inhibition achieved in mice that survived the treatment and are presented with the toxicity data for each group of animals. These data, however, suggest that prolonged schedules of administration are more efficacious in these models.

Prolonged administration schedules

The relationship between the duration of drug administration and the response was further examined in two moderately responsive xenografts, Rh12 rhabdomyosarcoma and VRC_5 colon adenocarcinoma. In the Rh12 experiment, mice received three 5-day $[(dx5)_3]$ couses of oral topotecan (2 or 1.75 mg/kg per dose) or a lower dose (1.25 mg/kg per administration) for up to 20 courses. The results are presented in Fig. 3. The administration of topotecan p.o. for three courses caused some complete regressions, although virtually all of the tumors recurred. In contrast, low-dose prolonged administration resulted in complete regression of all tumors without regrowth. In a separate experiment,

Table 5. Prolonged schedules of parenteral topotecan administration

Tumor	Dose[a]	Time to 4X ± SD	Growth delay (days)	T/C (min)	% Response[d]			Response ranking	Toxicity (deaths/ totals)
					PR	CR	C		
HC_1	0	25.8± 7.7							
	1.5	76.3± 2.4	50.5*	0.08 (34)	90	10	0	+++	0/7
	2.0	77.8± 9.9	52.0*	0.18 (34)	60	0	0	+++	0/7
GC_3	0	25.9± 6.6							
	2.0	40.3±23.8	14.4	0.83 (27)	8	0	0	+	0/7
VRC_5	0	16.1± 4.1							
	1.5	49.1±22.6	33.0*	0.10 (31)	66	33	33	+++	1/7
	2.0	44.4±23.3	28.3*	0.08 (27)	86	43	29	+++	0/7
SJC_3B	0	24.5± 8.9							
	1.75	46.0± 6.1	21.5*	0.22 (22)	7	0	0	+	0/7
	2.0	50.4± 9.4	25.9*	0.13 (22)	0	0	0	++	3/7
Rh18	0	17.9± 3.4							
(advanced)	2.0	39.0± 9.6	21.1*	0.11 (22)	83	0	0	++	2/7
Rh18/VCR	0	23.0± 3.0							
	2.0	38.8±17.1	15.8	0.09 (27)	100	17	17	++++	0/6
Rh28	0	17.8± 9.3							
	1.0	83.9± 0.3	66.1*	0.02 (36)	100	100	84	++++	1/7
	1.5	76.2± 8.1	58.4*	0 (35)	100	100	66	+++++	0/6
Rh30	0	11.8± 6.1							
	1.0	79.3± 7.4	67.5*	0 (34)	100	90	70	++++	1/7
	1.5	79.4± 5.6	73.8*	0 (34)	100	100	100	++++++	3/7
IRS68	0	14.6± 4.5							
	2.0	33.8± 8.6	19.2*	0.06 (32)	100	87	63	++++	3/7
OS2	0	20.8± 4.3							
	1.25	58.3±16.4	55.5*	0.09 (29)	75	50	50	+++	2/6
	1.75	52.2±12.1	49.4*	0.04 (29)	100	75	75	++++	1/7
	2.0	61.3±16.6	58.5*	0.07 (29)	100	75	75	++++	3/7

[a] Dose: mg/kd (daily × 5) for 3 courses i.p.; for descriptions of other parameters, see Table 2
* Significantly different from control values ($P < 0.05$)

complete regression of all Rh12 tumors was achieved at a daily dose of 1 mg/kg for 20 courses (data not shown).

The activity of i.p. administration (3 courses at 1.5 mg/kg per dose) and of 20 weeks of p.o. administration (1 mg/kg per dose) in VRC_5 colon adenocarcinoma xenografts is presented in Fig. 4. In VRC_5-bearing mice, the MTD (1/7 deaths) was 1.5 mg/kg for i.p. administration, whereas oral dosing was well tolerated. In this experiment, prolonged p.o. administration was more efficacious than i.p. administration for three courses. Three courses of i.p. topotecan (1.5 mg/kg per dose) was equi-effective with oral dosing using the same schedule (cf. Fig. 3, 1.75 mg/kg per dose).

Responses of osteosarcomas

Topotecan was evaluated against three lines of osteosarcoma. When given by oral gavage for three courses, topotecan (1.0–2.0 mg/kg per dose) caused significant growth inhibition in OS2 and OS9 xenografts, and complete regression of all OS33 tumors was achieved when mice were given 17 courses of topotecan p.o. (1.0 or 1.5 mg/kg per dose; Fig. 5). The response of OS2 xenografts in mice receiving three courses of i.p. topotecan (1.75 and 1.25 mg/kg per dose) is also shown in Fig. 5.

Cross-resistance to topotecan

Topotecan was evaluated in two tumors selected in vivo for resistance to VCR (Rh12/VCR and Rh18/VCR) and against a subline of Rh28 selected for primary resistance to L-PAM. Rh28/L-PAM is cross-resistant to VCR [15] and to VP-16 (unpublished data). Topotecan showed similar activity against VCR-resistant sublines and parental rhabdomyosarcomas (Fig. 6). In contrast, Rh28/L-PAM was considerably less sensitive to topotecan than were parental Rh28 tumors (Fig. 6).

Pharmacokinetics

Topotecan plasma concentrations exceeded the limit of assay sensitivity in both the p.o. and the i.p. group for the duration of the study (8 h). The plasma topotecan concentrations declined biexponentially in both groups. Figure 7 shows the mean (± SEM) topotecan concentration-time

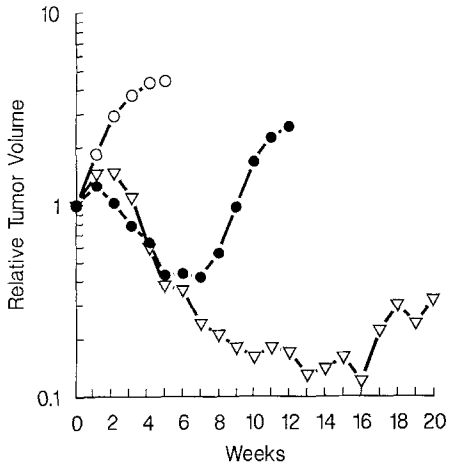

Fig. 4. Responses of VRC_5 colon adenocarcinoma xenografts to i.p. or prolonged p.o. administration of topotecan. ○, Control; ●, 1.5 mg/kg per dose given i.p. on a $(dx5)_3$ schedule; ▽, 1.0 mg/kg per dose given p.o. on a $(dx5)_{20}$ schedule. Each curve shows the mean response of 12 or 14 tumors

curves obtained following p.o. and i.p. administration. Pharmacokinetic parameters for each dosing route are summarized in Table 6.

Following i.p. administration, the volume of the central compartment (V_c) was 10.1 l/m^2 and the volume of distribution at steady state (Vd_{ss}) was 18.8 l/m^2. The apparent V_c and Vd_{ss} values obtained following oral dosing were 129.1 and 221.4 l/m^2, respectively.

Discussion

One criterion for the selection of a new compound prior to extensive testing in vivo requires that resistance not segregate with the typical MDR phenotype. Although the clinical significance of accelerated drug efflux via the *mdr* 1 gene product (P-glycoprotein) is presently unknown, overexpression of *mdr* 1 in childhood tumors has been reported [9]. Virtually all tumors of childhood are treated with drugs associated with the MDR phenotype (VCR, actinomycin D, doxorubicin, and frequently VP-16). Hence, it is probable that a new agent would demonstrate marginal activity in a phase II evaluation in which resistance might be mediated by this mechanism and would consequently generate insufficient enthusiasm for further development. There are also compelling data that demonstrate relatively high levels of expression of *mdr* 1 in colon adenocarcinomas and in xenografts from these [9, 15]. In the present study, topotecan exerted similar activity against $KBCh^R8$-5 and parental KB3-1 cells in vitro and hence fulfilled the criteria for subsequent in vivo evaluation.

Topotecan was subsequently evaluated against colon adenocarcinomas and rhabdomyosarcoma xenografts using an intermittent schedule of administration (q4dx4 i.p.). In the colon tumors derived from adults, topotecan caused significant growth inhibition (but no partial regression) in the VRC_5 line but did not show significant activity against the GC_3 or HC_1 xenografts. On the same dose schedule, topotecan caused partial and complete regressions in the majority of mice bearing Rh12, Rh18, and Rh28 rhabdomyosarcomas. Thus, topotecan demonstrated some activity against colon xenografts but showed significantly better activity against rhabdomyosarcomas.

As inhibitors of topoisomerase I appear to be highly cycle-phase-specific [2], prolonged administration schedules were considered to be an important determinant of cytoxicity, particularly for tumor-cell populations that have long cell-cycle times and low growth fractions. To test this possibility, we next examined the efficacy of topotecan given by oral gavage chronically over 3 weeks. The maxi-

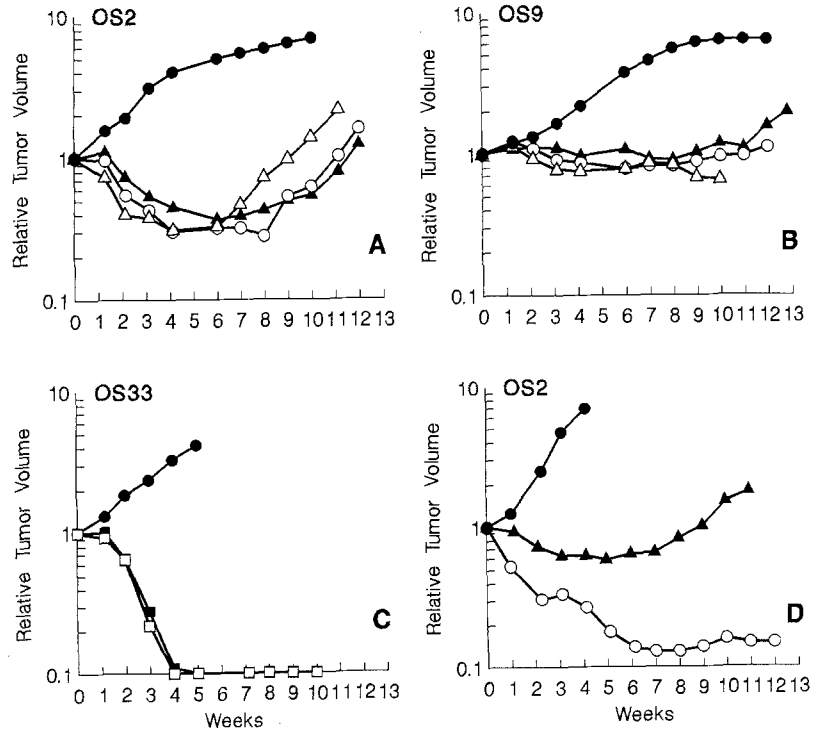

Fig. 5 A–D. Sensitivity of osteosarcoma xenografts to topotecan. **A** OS2. **B** OS9. Topotecan was given p.o. on a $(dx5)_3$ schedule – ●, control; △, 2 mg/kg per dose; ○, 1.5 mg/kg per dose; ▲, 1.0 mg/kg per dose. **C** OS33-bearing mice treated on the same schedule for 20 courses – ●, control; □, 1.5 mg/kg er dose; ■, 1.0 mg/kg per dose. **D** OS2 bearing mice treated with i.p. topotecan on a $(dx5)_3$ schedule – ●, control; ○, 1.75 mg/kg per dose; ▲, 1.25 mg/kg per dose. Each curve shows the relative tumor volume for groups of 12 or 14 tumors per group

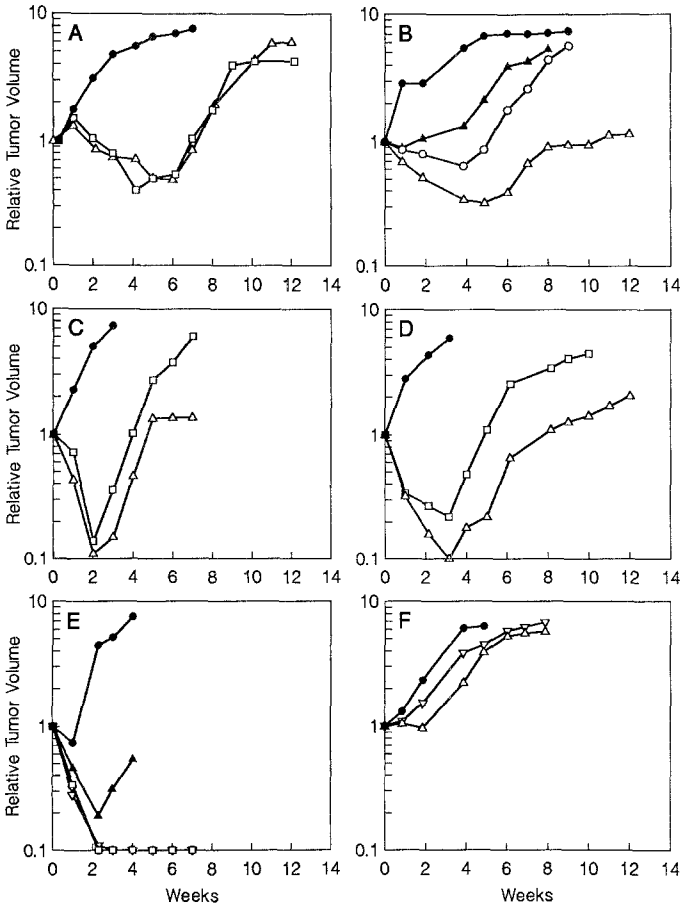

Fig. 6 A–F. Efficacy of oral topotecan against parental cell lines and VCR- or L-PAM-resistant sublines. **A** Rh12, **B** Rh12/VCR. **C** Rh18. **D** Rh18/VCR. **E** Rh28. **F** Rh28/LPAM. Topotecan was given by oral gavage on a $(dx5)_3$ schedule. ●, Control; △, 2 mg/kg per dose; □, 1.75 mg/kg; ○, 1.5 mg/kg per dose; ▲, 1.0 mg/kg per dose. ▽, Single i.p. injection of 13 mg/kg L-PAM. Each curve shows the relative tumor growth for 12 or 14 tumors per group

Table 6. Summary of pharmacokinetic parameters

Parameter	Mouse		Human[a]
	Route of administration		
	p.o.	i.p.	
C_{max} (ng/ml)	41.7	522.8	NA
T_{max} (h)	0.25	NA	NA
Cl_s (l h^{-1} m^{-2})	NA	20.0	20–125
Cl_{oral} (l h^{-1} m^{-2})	72.5	NA	NA
k_a (h^{-1})	13.9	NA	NA
$t_{1/2}\alpha$ (h)	0.55	0.29	0.06 to 0.15
$t_{1/2}\beta$ (h)	2.8	2.5	1.08 to 3.1
$AUC_{0\rightarrow\infty}$ (ng h ml^{-1})	91.3	288.8	NA

C_{max}, Maximal plasma concentration; T_{max}, time of maximal plasma concentration; Cl_s, systemic clearance; Cl_{oral}, apparent oral clearance; k_a, absorption rate constant; $t_{1/2\alpha}$, alpha half-life; $t_{1/2\beta}$, beta half-life; $AUC_{0\rightarrow\infty}$, area under the concentration-time curve from 0 to infinity; NA, not available ●

[a] From [28, 33–35]

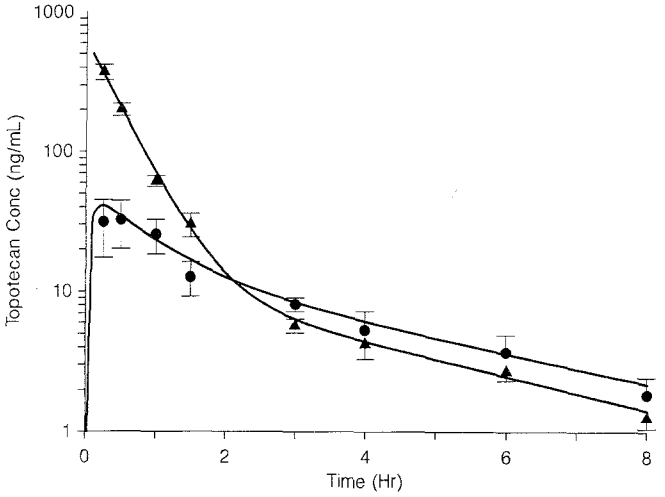

Fig. 7. Pharmacokinetic analysis of oral and i.p. administration of topotecan. Mean (± SEM) plasma topotecan concentration-time plots for both oral (●) and i.p. (▲) administration were generated from the respective compartmental parametric estimates

mal dose used in these experiments was 2 mg/kg daily for 5 days for 3 consecutive weeks. Toxicity, manifested by weight loss, and lethality were minimal. Thus, this dose may not be the MTD for this regimen. It is noteworthy that when given chronically, topotecan caused partial regression of HC_1, VRC_5, and SJC_3B tumors as well as significant growth inhibition in these xenografts and in SJC_3A tumors. The efficacy of a prolonged schedule of administration was even more evident against the rhabdomyosarcoma lines. Complete regression of Rh28, Rh30, IRS56, and IRS68 were achieved at the highest dose level, whereas this schedule was approximately equi-effective with, albeit less toxic to, the q4dx4 administration schedule in Rh12 and Rh18 xenografts. It is noteworthy that there was no evidence of an acute dose-response relationship in these experiments (see Fig. 4).

Consequently, we examined the effect of very prolonged administration schedules against specific xenograft lines (Rh12, VRC_5, OS33). The administration of low-dose oral topotecan (1.0–1.5 mg/kg per dose) for up to 20 weeks was nontoxic but caused complete regression of all Rh12 and OS33 tumors and partial regressions of all VRC_5 colon adenocarcinomas. These results suggest that in tumors in which topoisomerase I can be inhibited, prolonged administration schedules may determine the magnitude of the tumor response. Topotecan also demonstrated significant activity against three lines of osteogenic sarcoma, causing growth inhibition in OS9 and significant regression of OS2 and OS33 xenografts when topotecan was given optimally. Although only limited single-agent data are available for these xenografts, topotecan has proved to be significantly more efficacious than either high-dose methotrexate with leucovorin rescue [31] or VCR (unpublished data).

Parenteral (i.p.) injection of topotecan appears to be equally active in albeit significantly more toxic to, tumor-bearing mice as compared with its oral administration on the same schedule. Our pharmacokinetic studies showed that topotecan concentrations following i.p. injection were higher than those resulting from p.o. administration for 2 h after treatment, but thereafter the p.o. concentrations were slightly higher. This leads one to speculate that high peak concentrations may be associated with toxicity and that daily i.p. administration at lower doses may have greater efficacy. In these studies, the oral bioavailability was approximately 30%. Currently, no data are available to describe oral bioavailability for humans.

However, it is the schedule rather than the route of administration that appears to be important. Giovanella et al. [10] also used a protracted schedule for the administration of 20(RS)-9-amino-camptothecin and reported curative activity against three colon-tumor xenografts when the drug was given subcutaneously. Our data demonstrate significant activity for topotecan, although regressions were seen in only one of seven tumor lines. In part, this may have been due to the initiation of treatment against more advanced tumors. Treatment of advanced VRC_5 tumors with three courses of topotecan (p.o. or i.p.) caused partial regression of approximately 80% of the tumors and complete regression of 20% without resulting in obvious toxicity. It was therefore deemed likely that the response could be increased, as in the Rh12 and OS33 xenografts, by extending the treatment period. As shown in Fig. 6, 20 courses of topotecan were considerably more effective than three courses against VRC_5 xenografts. The activities of topoisomerase I have not yet been determined, but such studies may be informative if sensitivity to camptothecin analogs is related to the level or activity of this enzyme, as has been proposed elsewhere [10].

The efficacy of topotecan in xenografts selected in vivo for VCR resistance was examined in Rh12/VCR and Rh18/VCR tumors. These tumors are cross-resistant to doxorubicin and VP-16 and have detectable levels of *mdr*1 transcripts [16]. Against these tumors, topotecan exhibited activity essentially identical to that exerted against the respective parental tumor lines. Thus, in this limited series, no cross-resistance was demonstrated in vivo or in vitro against the MDR KB-Ch^R8-5 cell line. In contrast, cross-resistance was found in Rh28/L-PAM, a xenograft selected in vivo for resistance to L-PAM. The mechanism for L-PAM resistance is unknown, but this tumor is also cross-resistant to VCR and VP-16.

In summary, topotecan has significant activity against a panel of colon adenocarcinomas and causes regression of advanced rhabdomyosarcomas. Our data support the use of prolonged schedules of administration, which in the mouse can be well tolerated for up to 20 weeks. Topotecan was equally active against tumors selected for VCR resistance and may hence be effective against tumors exhibiting an MDR phenotype. The results of the present study indicate that due to its novel target, this agent may have significant activity against a spectrum of human malignancies and, in particular, may have a significant impact on the treatment of childhood tumors such as rhabdomyosarcoma and osteogenic sarcoma.

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