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Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors

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Abstract The efficacy of protracted schedules of therapy of the topoisomerase I inhibitors 9-dimethylaminomethyl-10-hydroxycamptothecin (topotecan) and 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin (irinotecan; CPT-11) were evaluated against a panel of 21 human tumor xenografts derived from adult and pediatric malignancies. Tumors included eight colon adenocarcinomas, representing an intrinsically chemorefractory malignancy, six lines derived from childhood rhabdomyosarcoma (three embryonal, three alveolar) representing a chemoresponsive histiotype, sublines of rhabdomyosarcomas selected in vivo for resistance to vincristine and melphalan, and three pediatric brain tmors. All tumors were grown at the subcutaneous site. Topotecan was administered by oral gavage 5 days per week for 12 consecutive weeks. The maximum tolerated dose (MTD) was 1.5 mg/kg per dose. Irinotecan was given by i.v. administration daily for 5 days each week for 2 weeks [(d × 5)2] (one cycle of therapy), repeated every 21 days. The MTD for three cycles was 10 mg/kg per dose. Treatment was started against advanced tumors. Topotecan caused a high frequency of objective regressions in one of eight colon tumor lines, whereas irinotecan caused complete regressions (CR) of all tumors in three colon lines and a high frequency of CRs in three additional lines. Both drugs demonstrated similar activity against rhabdomyosarcoma xenografts. Topotecan caused CR of all tumors in four of six lines, and irinotecan in five of six lines evaluated. Both agents retained full activity against tumors selected for primary resistance to vincristine, but only irinotecan retained activity against a tumor selected for primary resistance to melphalan. Both agents demonstrated good activity against brain tumor xenografts with irinotecan causing CR in two of three lines and topotecan inducing CR in one of three lines. Results indicate that low-dose protracted schedules of daily administration of these topoisomerase I inhibitors is either equi-effective or more efficacious than more intense shorter schedules of administration reported previously.

Key words CPT-11 · Irinotecan · Topotecan · Camptothecin · Topoisomerases · Xenografts

Introduction

The nuclear enzyme topoisomerase I relaxes supercoiled DNA and appears to be important for semiconservative replication of double-helical DNA, transcription, and chromosomal decondensation [1,2], and represents a novel target for cytotoxic agents. The antitumor activity of 20(S)-camptothecin, a plant alkaloid isolated from *Camptotheca acuminata*, was studied 20 years ago [3]. Camptothecin has been evaluated as the sodium salt, but has been found to be ineffective in patients with advanced disseminated melanoma or gastrointestinal malignancies [4, 5]. Other studies in China, however, have demonstrated activity of 10-hydroxycamptothecin in the treatment of head and neck and bladder cancers (reviewed in reference 2). Unpredictable and severe toxicities include myelosuppression,

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vomiting, diarrhea, and hemorrhagic cystitis that resulted in discontinuation of the clinical trial of sodium camptothecin. Recently, Giovanella et al. [6] reported curative activity of 9-amino-20(*S*)-camptothecin (9AC) against xenografts of early stage colon adenocarcinoma following administration of drug by subcutaneous (s.c.) implant, and Pantazis et al. [7] also reported that the camptothecin, 9AC and (9-nitro-20(*S*)-camptothecin), caused complete regression of BRO human melanoma xenografts.

Because of their insolubility in aqueous vehicles, extensive studies have identified more soluble and active camptothecin analogues. The water-soluble analogue, 9-dimethylaminomethyl-10-hydroxycamptothecin (topotecan) has been shown demonstrate broad-spectrum activity against rodent tumor models [2], and significant therapeutic activity against some human colon adenocarcinoma xenografts [8]. This suggests that inhibitors of topoisomerase I may have efficacy against malignancies generally regarded as chemorefractory. In addition, topotecan has curative activity against xenografts derived from childhood rhabdomyosarcomas [8], and significant activity against xenografts derived from childhood and adult brain tumors [9]. Topotecan is currently undergoing phase I/II clinical trials in adults and children. Irinotecan, a water-soluble prodrug, has ben shown to demonstrate broad-spectrum activity against experimental tumor models [10–15] and against P-glycoprotein-expressing multidrug resistant (Pgp-MDR) cell lines [16]. The dose-limiting toxicity of irinotecan given as a single i.v. administration to humans is severe myelosuppression [17]. In this phase I trial objective responses were observed in previously treated patients with sarcoma of the stomach, melanoma, colon adenocarcinoma and non-small-cell lung (NSCLC) cancer. Irinotecan administered weekly by short infusion has been shown to demonstrate activity against refractory or relapsed SCLC [18] and responses have been measured in non-Hodgkin's lymphoma, acute lymphocytic leukemia [19], and NSCLC [20] with differing schedules. These data suggest quite broad-spectrum clinical activity for irinotecan, and combination phase I trials with cisplatin [21, 22], vindesine [23], 5-fluorouracil [24] and etoposide [25] have been reported.

While clinical trials are focused upon phase II development and evaluation of topoisomerase I inhibitors in combination with other cytotoxic agents, there is relatively little information regarding the optimal scheduling of this class of antitumor agent. In previous studies [8, 9] we have demonstrated the therapeutic advantage of low-dose protracted schedules of topotecan in the treatment of several tumors. Here we report the evaluation of protracted schedules of low-dose topotecan and irinotecan against a panel of 21 xenografts derived from adult and childhood malignant tumors.

Materials and methods

Immune deprivation of mice

Female CBA/CaJ mice (Jackson Laboratories, Bar Harbor, Me.), 4 weeks of age, were immune-deprived by thymectomy, followed 3 weeks later by whole-body irradiation (950 cGy) using a ^{137}Cs source. Mice received 3×10^6 nucleated bone marrow cells within 6–8 h of irradiation [26]. Tumor pieces of approximately $3mm^3$ were implanted in the space of the dorsal lateral flanks of the mice to initiate tumor growth. Tumor-bearing mice were randomized into groups of six or seven prior to initiating therapy.

Tumor lines

Each of the colon and rhabdomyosarcoma xenografts have been described previously [27–38]. Colon tumors designated SJC were from young patients (11–26 years). Brain tumors D283 and DAOY, both medulloblastomas have been described previously [39]. The glioblastoma multiforme, SJ-GBM2, was derived at autopsy from a 5-year-old female patient. For chemotherapy studies, all tumors were used within 27 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, development of resistance and characteristics of xenografts have been presented previously [8,15, 30–38].

Growth inhibition studies

Mice bearing bilateral subcutaneous tumors each received the agent when tumors were approximately 0.20–1 cm in diameter. The procedures have been reported previously [8]. Briefly, two perpendicular diameters were determined at 7-day intervals using digital Vernier calipers interfaced with a Dell 486/66 computer. Tumor volumes were calculated assuming tumors to be spherical using the formula $[(\pi/6) \times d^3]$, where d is the mean diameter [34].

Formulation and administration

Irinotecan (clinical formulation) was diluted in sterile saline, and administered i.v. (0.1 ml/10-g body weight) daily for 5 days on two consecutive weeks followed by a 7-day rest period, referred to as one cycle of therapy. Mice received a maximum of three cycles (designated $[(d \times 5)2]3$) limited by our ability to give a greater number of i.v. injections after this time. Where appropriate, control mice received vehicle (0.26 ml 70%w/w sorbital, 0.9 mg lactic acid per ml, pH 3.9). Preliminary studies indicated that whereas topotecan administered at 2 mg/kg per dose was tolerated for three consecutive 5-day courses, extending the number of courses resulted in toxicity. Consequently, the dose was reduced 25%, to 1.5 mg/kg per dose, and was tolerated for at least 12 consecutive courses of treatment. The maximum body weight loss for groups of mice treated was 6–13%. Similarly, irinotecan was tolerated at 10 mg/kg per dose for three cycles, and resulted in a mean body weight loss for groups of mice 10–17% in different experiments. Higher daily doses (i.e. 15 mg/kg per dose) resulted in higher levels of lethality ($> LD_{10}$).

Topotecan was dissolved in water for oral gavage (0.05 ml/10-g body weight) and administered daily 5 days per week for 12 consecutive weeks. Irinotecan was generously provided by Dr. K. Terada, Yakult Co. Ltd., Japan, or Dr. A. Mathieu-Boué, Laboratoire Roger Bellon (Paris). Topotecan was generously provided by Dr. Randall K. Johnson, SmithKline Beecham (King of Prussia, Philadelphia, Pa.).

Statistical Analysis

The results of individual tumor inhibition studies were analyzed with one-way analysis of variance, using the number of days to reach four 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 four times the original volume was assigned a default value of the maximum duration of the study.

The percent of tumors showing partial and/or complete regression and any regrowth were calculated for the individual tumor lines as described previously [26]. For individual tumors partial response (PR) was defined as a volume regression > 50% but with measurable tumor at all times. Complete regression (CR) was defined as disappearance of measurable tumor mass at some point after initiating therapy. For data summaries (Tables 7 and 8) it was required that all tumors within a treatment group fulfilled the minimum criteria for PR or CR for the 'group response' to be so defined.

Results

Colon adenocarcinomas

Previous studies with topotecan [8] have demonstrated that prolonged daily administration, 5 days per week for three consecutive weeks, has greater therapeutic efficacy than drug given in a more intense schedule (every 4 days for 4 administrations). Similarly, irinotecan given for two consecutive 5-day courses was more effective than a single administration [15]. Consequently, we evaluated the efficacy of protracted schedules of daily administration of low doses of irinotecan and topotecan. Tumor responses to topotecan and irinotecan are summarized in Tables 1 and 2, respectively.

Given daily 5 days per week for 12 weeks, topotecan (1.5 mg/kg per dose) caused a high frequency of objective regressions in only VRC_5 tumors, but caused significant growth inhibition in an additional four lines. In contrast, irinotecan caused CR of all HC_1, VRC_5 and SJC_8 xenografts when administered at the MTD (10 mg/kg per dose). Irinotecan also induced a high frequency of CR and PR in three additional tumors (Table 2). Irinotecan showed activity over a fourfold dose range, as demonstrated for HC_1 xenografts (Fig. 1). This agent also demonstrated significant activity against SJC_8 colon tumors that are intrinsically resistant to topotecan, (Fig. 2).

Rhabdomyosarcomas

Both topotecan and irinotecan demonstrated significant activity against xenografts derived from childhood rhabdomyosarcomas. Topotecan caused CR of all tumors in four of six lines tested. However, there was a marked decrease in activity at the lowest dose level used (0.5 mg/kg per dose) in both Rh28 and Rh30 xenografts (Table 3). Irinotecan caused CR in five of six rhabdomyosarcomas, with a high frequency of CR in

Table 1 Responses of colon carcinomas to protracted schedules of topotecan (*PR* ≥ 50% regression, *CR* complete regression, *MCR* maintained CR at the end of the experiment, week 12)

Tumor	Dose (mg/kg)	Days to 4× ± SE[a]	Growth delay (days)	Regressions (%)		
				PR	CR	MCR
HC_1	0	29.0 ± 3.5				
	1.5	69.7 ± 3.6	40.7	7	0	0
GC_3	0	24.2 ± 2.0				
	1.0	24.3 ± 1.8	0.1	0	0	0
	1.5	31.1 ± 2.9	6.8	0	0	0
VRC_5	0	9.5 ± 2.5				
	0.5	> 84	> 75	21	0	0
	1.0	> 84	> 75	86	14	14
	1.5	> 84	> 75	29	71	71
ELC_2	0	30.2 ± 1.9				
	1.0	73.1 ± 5.5	43.0	0	0	0
	1.5	80.5 ± 0	50.3	0	0	0
SJC_2	0	19.0 ± 1.6				
	1.5	31.9 ± 1.9	12.9	0	0	0
SJC_3A	0	22.8 ± 2.9				
	1.5	60.4 ± 9.3	37.6	25	12.5	12.5
SJC_3B	0	31.0 ± 2.2				
	1.0	24.9 ± 0.6	− 6.1	0	0	0
	1.5	58.9 ± 3.7	27.9	29	0	0
SJC_8	0	28.0 ± 3.2				
	1.0	42.3 ± 2.9	14.3	0	0	0
	1.5	63.0 ± 2.7	35.0	0	0	0

[a]Days required for tumors to grow to four-times their value at the start of treatment

Table 2 Responses of colon carcinomas to protracted schedules of irinotecan (*PR* ≥ 50% regression, *CR* complete regression, *MCR* maintained CR at the end of the experiment, week 12)

Tumor	Dose (mg/kg)	Days to 4× ± SE[a]	Growth delay (days)	Regressions (%) PR	CR	MCR
HC_1	0	31.5 ± 2.9				
	2.5	> 84	> 52	25	75	66
	5.0	> 84	> 52	17	83	83
	10.0	> 84	> 52	0	100	100
GC_3	0	25.2 ± 3.2				
	2.5	77.6 ± 1.9	52.4	58	17	17
	5.0	77.3 ± 2.3	52.1	36	46	36
	10.0	> 84	> 55	75	33	33
VRC_5	0	13.4 ± 1.1				
	2.5	> 84	> 71	72	28	28
	5.0	> 84	> 71	0	100	100
	10.0	> 84	> 71	0	100	100
ELC_2	0	28.7 ± 2.2				
	5.0	70 ± 4.3	41.3	0	0	0
	10.0	> 84	> 55	0	0	0
SJC_2	0	23.9 ± 1.9				
	5.0	72.3 ± 4.2	48.4	33	17	0
	10.0	75.5 ± 1.8	51.6	21	71	7
SJC_3A	0	28.3 ± 1.8				
	5	> 84	> 56	36	57	14
	10	> 84	> 56	21	79	29
SJC_3B	0	19.5 ± 1.4				
	5.0	> 84	> 64	0	100	33
	10.0	> 84	> 64	10	75	75
SJC_8	0	23.4 ± 2.4				
	5	> 84	> 61	0	100	100
	10	> 84	> 61	0	100	100

[a]See footnote to Table 1

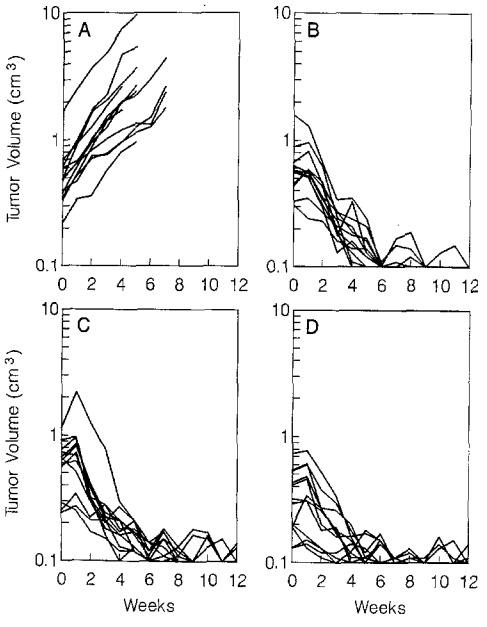

Fig. 1 A–D Responses of HC_1 colon adenocarcinoma xenografts to irinotecan. Mice bearing advanced s.c. tumors were treated i.v. with three cycles of irinotecan. **A** Controls (vehicle), **B**, **C**, and **D** irinotecan 10, 5, and 2.5 mg/kg per dose, respectively. Each curve represents the growth of an individual tumor

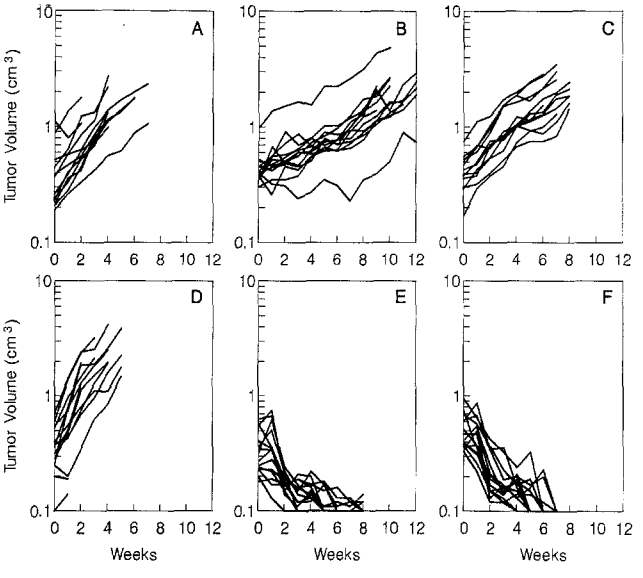

Fig. 2 A–F Sensitivity of SJC_8 colon xenografts to topotecan (**A–C**) and irinotecan (**D–F**). Mice received vehicle only (**A**, **D**), topotecan orally (**B**, **C**) for 12 consecutive 5-day courses, or irinotecan i.v. for three cycles (**E**, **F**). **B**, **C** 1.5 and 1.0 mg/kg per dose topotecan, respectively. **E**, **F** 10 and 5 mg/kg per dose irinotecan, respectively. Each curve represents the growth of an individual tumor

Table 3 Responses of rhabdomyosarcoma to protracted schedules of topotecan (*PR* ≥ 50% regression, *CR* complete regression, *MCR* maintained CR at the end of the experiment, week 12)

Tumor	Dose (mg/kg)	Days to 4× ± SE[a]	Growth delay (days)	Responses (%) PR	CR	MCR
Rh12	0	35.6 ± 7.0				
	1.5	> 84	> 48	18	82	82
Rh18	0	24.5 ± 0.4				
	1.5	65.3 ± 5.0	41	0	17	17
Rh28	0	21.5 ± 3.9				
	0.5	31.5 ± 1.1	10	0	0	0
	1.0	> 84	> 53	0	100	100
	1.5	> 84	> 53	0	100	100
Rh30	0	18.1 ± 1.1				
	0.5	29.5 ± 4.2	11	7	0	0
	1.0	> 84	> 66	0	100	100
	1.5	> 84	> 66	0	100	100
IRS56	0	35.9 ± 2.9				
	0.5	> 84	> 48	0	100	100
	1.0	> 84	> 48	0	100	100
	1.5	> 84	> 48	0	100	100
IRS68	0	23.5 ± 3.8				
	1.0	> 84	> 60	0	100	100
	1.5	> 84	> 60	0	100	100

[a]See footnote to Table 1

Table 4 Responses of rhabdomyosarcoma to protracted schedules of irinotecan (*PR* ≥ 50% regression, *CR* complete regression, *MCR* maintained CR at the end of the experiment, week 12)

Tumor	Dose (mg/kg)	Days to 4× ± SE[a]	Growth delay (days)	Responses (%) PR	CR	MCR
Rh12	0	27.6 ± 2.5				
	5	> 84	> 57	15	85	85
	10	> 84	> 57	21	79	79
Rh18	0	20.3 ± 2.5				
	2.5	> 84	> 63	14	86	79
	5	> 84	> 63	0	100	100
	10	> 84	> 63	0	100	100
Rh28	0	16.5 ± 1.7				
	5	> 84	> 67	0	100	100
	10	> 84	> 67	0	100	100
Rh30	0	19.0 ± 1.4				
	2.5	> 84	> 65	0	100	100
	5	> 84	> 65	0	100	100
	10	> 84	> 65	0	100	100
IRS56	0	28.9 ± 3.1				
	2.5	> 84	> 55	0	100	100
	5	> 84	> 55	0	100	100
	10	> 84	> 55	0	100	100
IRS68	0	32.7 ± 4.1				
	5	> 84	> 51	0	100	100
	10	> 84	> 51	0	100	93

[a]See footnoe to Table 1

Rh12 tumors. Of note was that irinotecan was highly active against Rh18 tumors that were only moderately sensitive to topotecan administered on this schedule, (Table 4).

Cross resistance to topoisomerase I inhibitors

Topotecan and irinotecan were evaluated in two tumors selected in vivo for resistance to vincristine

Table 5 Responses of in vivo drug-selected tumors to protracted schedules of topoisomerase I inhibitors (*PR* ≥ 50% regression, *CR* complete regression, *MCR* maintained CR at the end of the experiment, week 12)

Tumor	Dose (mg/kg)	Days to[a] 4× ± SE	Growth delay (days)	Responses (%) PR	CR	MCR
Topotecan						
Rh12/VCR	0	23.7 ± 0.3				
	1.0	74.0 ± 2.4	50	36	7	7
	15	> 84	> 60	14	86	86
Rh18/VCR	0	29.0 ± 0.4				
	1.0	63.5 ± 5.2	34	0	0	0
	1.5	78.8 ± 1.7	50	71	36	29
Rh28/LPAM	0	23.2 ± 3.5				
	0.5	29.4 ± 5.7	6	8	0	0
	1.0	45.0 ± 7.5	22	36	21	21
	1.5	33.0 ± 6.2	10	0	0	0
Irinotecan						
Rh12/VCR	0	24.5 ± 3.6				
	2.5	> 84	> 59	7	93	100
	5	> 84	> 59	0	100	93
	10	> 84	> 59	0	100	100
Rh18/VCR	0	27.5 ± 0.1				
	5	> 84	> 56	0	100	50
	10	> 84	> 56	0	100	100
Rh28/LPAM	0	23.2 ± 3.7				
	5	78.5 ± 4.3	55	21	79	64
	10	> 84	> 61	7	93	64

[a]See footnote to Table 1

(Rh12/VCR and Rh18/VCR) and against a subline of Rh28 selected for primary resistance to melphalan (L-PAM). Rh28/LPAM is also cross-resistant to vincristine [35] and etoposide [38]. Both agents had similar activity against vincristine sublines (Rh12/VCR and Rh18/VCR) and their respective parental tumors, (Table 5). Topotecan demonstrated little activity against Rh28/LPAM, whereas irinotecan caused CR in a high frequency of tumors (Fig. 3).

Responses of brain tumor xenografts

Previously it has been shown that topotecan has significant activity against several brain tumor xenografts implanted either s.c or intracranially (i.c.) in athymic nude mice [9]. To extend these studies, and to evaluate protracted therapy with topoisomerase I inhibitors, these agents were tested against an additional three tumor lines. As shown in Table 6, topotecan and irinotecan, respectively, caused CR in a high proportion of tumors in the three lines tested. Of particular note is the activity against SJ-GBM2 xenografts, derived from a heavily pretreated patient. This xenograft is resistant to agents such as vincristine, LPAM, doxorubicin and cyclophosphamide (data not shown)

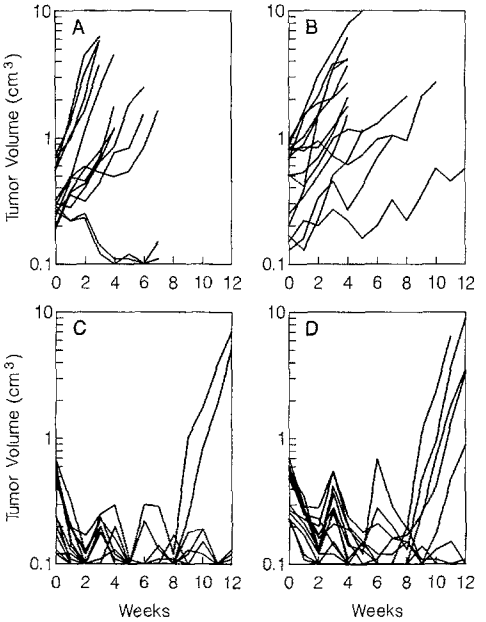

Fig. 3 A–D Responses of Rh28/LPAM xenografts to topotecan and irinotecan administered as low-dose protracted therapy. **A** Control (water orally). **B** Topotecan 1.5 mg/kg per dose orally, 5 days per week for 12 weeks. **C**, **D** Irinotecan 10 and 5 mg/kg, respectively, given for three cycles. Each curve represents the growth of an individual tumor

Table 6 Responses of brain tumor xenografts to protracted schedules of topotecan and irinotecan (*PR* ≥ 50% regression, *CR* complete regression, *MCR* maintained CR at the end of the experiment, week 12)

Tumor	Dose (mg/kg)	Days to 4× ± SE[a]	Growth delay (days)	Responses (%)		
				PR	CR	MCR
Topotecan						
D283	0	23.3 ± 0.3				
	1.0	> 84	> 61	71	29	29
	1.5	> 84	> 61	14	86	86
DAOY	0	21.0 ± 2.3				
	1.0	> 84	> 63	0	100	100
	1.5	> 84	> 63	0	100	100
SJ-GBM2	0	8.5 ± 1.0				
	1.0	65.9 ± 5.4	57	9	58	58
	1.5	> 84	> 75	21	79	79
Irinotecan						
D283	0	16.5 ± 1.0				
	5	73 ± 4.4	56	43	43	36
	10	> 84	> 63	0	100	100
DAOY	0	23.8 ± 3.2				
	5	> 84	> 60	30	70	70
	10	> 84	> 60	12	88	88
SJ-GBM2	0	8.55 ± 1.1				
	5	> 84	> 75	0	93	93
	10	> 84	> 75	0	100	100

[a]See footnote to Table 1

administered at the optimal dose and schedule (~ LD_{10}) to tumor-bearing mice (Fig. 4).

Discussion

Inhibitors of topoisomerase I appear to be highly S-phase specific [40], and vitro, cytotoxicity is a function of exposure time to drug above some critical concentration. Consequently, prolonged inhibition of topoisomerase I should be considered an important parameter in causing cytotoxicity in vivo. However, it remains to be determined whether exposure by continuous drug infusion offers the optimal differential between normal tissue toxicity and tumor cytotoxicity. Currently, topotecan is being investigated clinically in a number of different schedules including short (24-h), intermediate (72–120-h) and protracted (21-day) infusions, as well as bolus administration daily for 5 days or every 21 days. Scheduling of irinotecan has been less extensively investigated, it is usually administered every 7, 14 or 28 days. Alternatively, very high doses (400 to 600 mg/m^2) have been administered with loperamide used to control diarrhea [41]. As with all phase I studies, the objective has been to determine the maximal tolerated dose levels for a given schedule of administration. However, considering the mechanism and kinetics of drug action, such definition of maximal dose intensity may not necessarily be optimal for this class of antitumor agent.

In previous studies using xenograft models, we have approached scheduling of topoisomerase inhibitors differently. As a relatively brief exposure to topoisomerase I inhibitors is lethal to S-phase cells, we postulated that pulsing these drugs, by daily bolus administration, over a prolonged period may prove more efficacious, particularly in tumors that have a relatively low proportion of S-phase cells. Thus, rather than defining toxicity for intensive courses of therapy we have attempted to determine the efficacy of low doses of topoisomerase I inhibitors that would be tolerated when given over a period of 9–12 weeks. These studies indicated that irinotecan administered daily for two 5-day courses, and topotecan for three 5-day courses were more effective than more intensive schedules of therapy. Further, topotecan given for 20 consecutive 5-day courses was more efficacious than three courses. Consequently, in this study we evaluated more protracted therapy with topotecan and irinotecan against an extended panel of tumors.

Topotecan was administered by oral gavage 5 days per week for the duration of the experiments (12 weeks). Irinotecan was administered by i.v. injection 5 days for each of two consecutive weeks followed by an interval of 1 week without treatment, and this cycle was repeated an additional two times. Toxicity from either

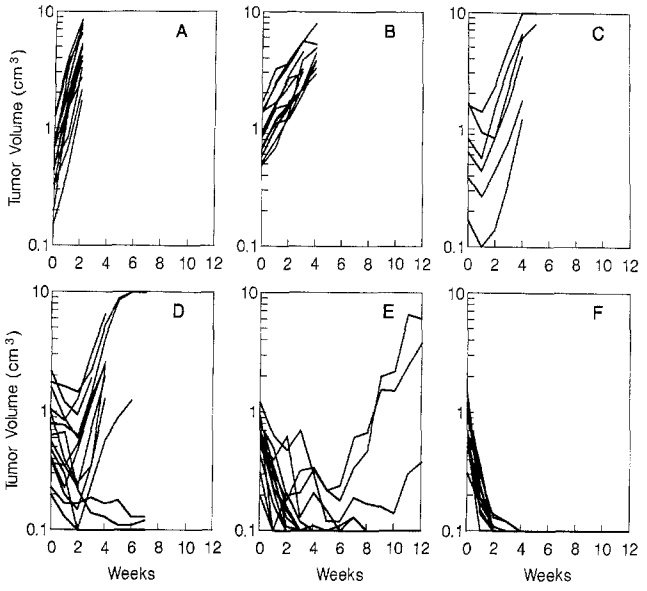

Fig. 4 A–F Responses of advanced SJ-GBM2 glioblastoma xenografts to chemotherapeutic agents. **A** Control (no treatment); **B** doxorubicin 10 mg/kg i.v every 7 days for 2 administrations vincristine 3 mg/kg i.p single administration; **D** melphalan 13 mg/kg i.p. single administration; **E** topotecan 1.5 mg/kg per dose orally, 5 days per week for 12 weeks; **F** irinotecan 10 mg/kg per dose i.v. for three cycles. Each curve represents the growth of an individual tumor

drug given at the highest dose level (1.5 mg/kg per dose for topotecan, 10 mg/kg per dose for irinotecan) was minimal with 3/182 deaths for topotecan and 1/156 for irinotecan. Maximum weight loss at the highest dose level of topotecan ranged from 6 to 13%, and for irinotecan from 8 to 17%. Thus, the toxicities were similar for the highest dose level of each agent. Higher dose levels were not tolerated using the schedules presented, although for topotecan up to 20 consecutive courses at 1.5 mg/kg per kg per dose have been given [8]. Similarly, additional cycles of irinotecan were not precluded by toxicity, but rather were limited because of the route of administration (i.v.).

The efficacies of both agents are summarized in Tables 7 and 8. Prolonged therapy with topotecan showed essentially similar activity against colon tumors to that obtained with 3 weeks of therapy [8]. Objective responses (PR, CR) in a high frequency were determined only in VRC_5 colon adenocarcinomas. In contrast, irinotecan appeared to be more effective given on this low-dose prolonged schedule against all of the colon adenocarcinomas except SJC_3A compared to previously reported results using the more intensive and toxic schedule (compare Table 8 with Table 4 of reference 15). Three cycles of irinotecan therapy resulted in CR of all tumors in three colon lines (HC_1, VRC_5, SJC_8). In addition irinotecan caused a high proportion of CR and PR in SJC_2, SJC_3A, SJC_3B, and GC_3 xenografts. Thus, in colon tumors HC_1, VRC_5, SJC_2, and SJC_8 irinotecan given at low doses over 9 weeks (total dose 300 mg/kg) was more effective than

Table 7 Efficacy of protracted schedules of topotecan. Topotecan was administered orally 5 days per week for 12 weeks–no growth inhibition, ± growth inhibition < 1 tumor volume doubling time (TD2), + growth inhibition ≥ 1 × TD2, ++ growth inhibition ≥ 2 × TD2, +++ growth inhibiion ≥ 3 × TD2, ++++ growth inhibition > 3 × TD2 with ≥ 50% regression, +++++ complete regression of all tumors, ++++++ CR of all tumors without regrowth during experimenal period, 12 weeks, *ND* not determined

	Dose (mg/kg)		
Colon tumors	1.5	1.0	0.5
HC_1	++	ND	ND
GC_3	–	–	ND
VRC_5	++++	++++	+++
ELC_2	+++	+++	ND
SJC_2	+	ND	ND
SJC_3A	+++	ND	ND
SJC_3B	+	–	ND
SJC8	++	±	ND
Rhabdomyosarcoma			
Rh12	++++	ND	ND
Rh18	+++	ND	ND
Rh28	++++++	++++++	–
Rh30	++++++	++++++	+
IRS56	++++++	++++++	++++++
IRS68	++++++	++++++	ND
Rh12/VCR	++++	+++	ND
Rh18/VCR	+++	++	ND
Rh28/LPAM	–	++	–
Brain tumors			
D283	++++	++++	ND
DAOY	++++++	++++++	ND
SJ-GBM2	++++	++	ND

40 mg/kg daily for 5 days on each of two consecutive weeks (400 mg/kg total dose).

Rhabdomyosarcoma xenografts were highly responsive to both topotecan and irinotecan given either on protracted or by more intensive schedules, consequently it is difficult to determine whether protracted schedules offer a significant advantage. Only Rh18 tumors were less responsive to protracted topotecan, as intensive treatment (d × 5)3 caused complete regressions [8]. However, for Rh28, Rh30, and IRS56 xenografts there were fewer tumors that regrew after CR using low-dose protracted treatment schedules. It is noteworthy that the efficacy of topotecan decreased very dramatically between dose levels of 1.0 mg/kg per dose and 0.5 mg/kg per dose. In both Rh28 and Rh30 tumors CR was obtained at 1.0 mg/kg per dose, but virtually no regressions and only minimal growth inhibition were measured at 0.5 mg/kg per dose (Table 3). These results may have value in defining a minimal effective concentration–time relationship for topotecan administered by daily oral gavage. Irinotecan was also highly active against the rhabdomyosarcoma xenografts, the protracted schedule being more effective in Rh12 tumors than the intensive schedule [15]. Both

Table 8 Efficacy of protracted schedules of irinotecan. Irinotecan was administered i.v. 5 days per week for 2 weeks followed by a 7-day rest period (one cycle), to a total of three cycles (for explanation of symbols, see Table 7)

	Dose (mg/kg)		
Colon tumors	10	5	2.5
HC_1	++++++	++++++	++++++
GC_3	+++	+++	+++
VRC_5	++++++	++++++	++++
ELC_2	+++	+++	ND
SJC_2	++++	+++	ND
SJC_3A	++++	++++	ND
SJC_3B	++++	++++	ND
SJC8	++++++	++++++	ND
Rhabdomyosarcoma			
Rh12	++++	++++	ND
Rh18	++++++	++++++	++++
Rh28	++++++	++++++	ND
Rh30	++++++	++++++	++++++
IRS56	++++++	++++++	++++++
IRS68	+++++	++++++	ND
Rh12/VCR	++++++	+++++	++++++
Rh18/VCR	++++++	+++++	ND
Rh28/LPAM	+++++	++++++	ND
Brain tumors			
D283	++++++	+++	ND
DAOY	++++	++++	ND
SJ-GBM2	++++++	+++++	ND

drugs retained activity against rhabdomyosarcomas selected in situ for vincristine resistance, as has been found with more intensive schedules of therapy. However, there was a significant difference between irinotecan and topotecan in the Rh28 tumor selected for resistance to melphalan (Rh28/LPAM). This tumor was completely resistant to topotecan administered over 12 weeks but highly sensitive to low-dose protracted schedules of irinotecan. Similar results have been reported using more intense schedules of drug administration [15]. Results from Rh28/LPAM xenografts, and other tumors that respond poorly to topotecan, but which are highly responsive to irinotecan (colon tumors HC_1, SJC_2, $SJC3_A$ and SJC_8) could indicate qualitative differences in these two agents that target topoisomerase I. The results indicate also that measurement of topoisomerase I levels or activity is unlikely to be predictive of response to these agents.

We have reported previously that topotecan demonstrates good activity against several pediatric and adult brain tumor xenografts [9]. In that study, topotecan was administered as two consecutive 5-day courses and caused regressions in both childhood and adult high-grade gliomas and two ependymoma xenografts growing at the s.c. site. Topotecan also increased the median survival of mice with i.c. implanted gliomas. The results presented here extend these data, and show very significant responsiveness of two medulloblastomas and a glioblastoma (SJ-GBM2) xenograft to topoisomerase I inhibitors. The result with SJ-GBM2 is encouraging as this xenograft is relatively resistant to other agents used in more intensive and toxic schedules (Fig. 4).

In summary, topotecan and irinotecan were administered at relatively low dose levels for protracted courses of therapy, and the responses of 21 xenografts examined. Prolonged daily administration of drugs was well tolerated and tumor responses were generally as good or superior to results with more intensive therapeutic regimens using these agents that we reported previously. Protracted low-dose therapy with topotecan did not increase the response rate in colon adenocarcinomas, but was highly efficacious in the rhabdomyosarcomas and brain tumors. Also, as the highest dose level used (1.5 mg/kg per dose) was tolerated for at least 20 consecutive courses [8], it is probable that results presented here may not reflect the optimal effect of topotecan. Low-dose protracted therapy with irinotecan was highly effective against virtually all tumors tested, and also may not represent the optimal effect. As irinotecan must first be activated by carboxyl esterase to form the active metabolite SN-38, administration of low doses may prevent potential saturation of this pathway. Further, it has been proposed that diarrhea associated with irinotecan administration may result from biliary excretion of SN-38 in patients who have a lower rate of formation of SN-38-glucoronide conjugate [42]. As conjugation of SN-38 may be rate limiting, the use of low-dose protracted therapy may offer some advantages in reducing gastrointestinal toxicity associated with this agent. Glucoronosyltransferase deficiency as well as capacity-limited activity has been related to toxicity of agents such as acetominophen [43, 44].

Our studies presented here, and previously reported results using topotecan [8] and irinotecan [15], indicate that increasing dose intensity beyond some critical point offers no advantage in causing tumor regression, but rather, in mice, leads to increased toxicity. Reducing dose intensity (by giving the same dose over a longer period) appears to offer some therapeutic advantage, with reduced host toxicity and equal or superior tumor responses. We consider that these results offer potential guidelines for the optimal use of topoisomerase I inhibitors. However, whether plasma pharmacokinetics for topotecan and irinotecan and its active metabolite, SN-38, similar to those obtained in mice using the schedules described, may be achieved in humans will be determined only by clinical studies. Studies using low-dose protracted schedules of topoisomerase I inhibitors are now being carried out to examine combinations with other cytotoxic agents in these xenograft models.

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