Anti-Cancer Drugs 1997, 8, pp. 313–322

Efficacy of oral irinotecan against neuroblastoma xenografts

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The efficacy of the topoisomerase I inhibitor, 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin (irinotecan, CPT-11), administered by oral gavage has been examined against a panel of six independently derived neuroblastoma xenografts. Irinotecan was administered either daily for 5 days on 12 consecutive weeks {(d × 5)12} or for 5 days on two consecutive weeks repeated every 21 days for 4 cycles {[(d × 5)2]4}. Given on the (d × 5)12 schedule the maximum tolerated dose (MTD) was 50 mg/kg. For intermittent scheduling {[(d × 5)2]4}, the MTD was 75 mg/kg, resulting in the same total dose being administered (3 g/kg) over the period of treatment. At the MTD for the 12 consecutive week schedule there were two of 42 toxicity related deaths, whereas intermittent scheduling at the MTD resulted in none of 42 deaths. The intermittent schedule {[(d × 5)2]4} was less toxic than therapy given (d × 5)12, as at the end of treatment mice weighed 92 ± 4% (SD; *n* = 6 experiments) and 81 ± 4% (SD; *n* = 6 experiments) of their body weight at the start of therapy, respectively. The latter schedule was associated with loose feces starting around week 8 of therapy, broken teeth and a high incidence of swelling of the orbital conjunctiva that developed late in the course of therapy. Given (d × 5)12, irinotecan caused complete regressions of all six neuroblastoma xenograft lines. Because mice tolerate significantly greater systemic exposure to SN-38 lactone than do patients (as determined by plasma AUC at the respective MTD), we evaluated the intermittent schedule of administration, reducing the dose/administration to determine the lowest dose levels that produced objective regressions of these neuroblastoma xenografts and determined the daily systemic exposure associated with these dose levels. In four lines examined objective responses were obtained at dose levels of 12.5 or 6.25 mg/kg. The daily plasma AUC exposures associated with minimum dose achieving response in NB1691 (12.5 mg/kg), NB1643 (6.25 mg/kg) and NBEB (12.5 mg/kg) for irinotecan lactone were 219, 152 and 653 ng-h/ml, respectively; and for SN-38 lactone were 704, 418 and 987 ng-h/ml, respectively. These results indicate that childhood neuroblastoma xenografts are highly sensitive to irinotecan given by oral administration and therapeutic activity is similar to i.v. irinotecan administered on similar schedules.

Key words: Irinotecan, neuroblastoma, xenograft.

Introduction

Topoisomerases are essential nuclear enzymes that function to resolve topological problems in DNA. Topoisomerase 1 relaxes supercoiled duplex DNA so that replication and transcription can proceed,[1,2] and is the target for camptothecin (CPT) antitumor agents.[3] CPT is a plant alkaloid obtained from the Chinese tree *Camptotheca acuminata*, and was shown to have significant antitumor activity *in vitro* and against experimental animal tumor models *in vivo*. In early clinical trials, where the more soluble sodium salt was administered, however, the response rates were poor and the agent exhibited unpredictable toxicities. Because of the insolubility of CPT in aqueous vehicles, extensive studies have identified more soluble and active CPT analogs. We and others have reported that 9-dimethylaminomethyl-10-hydroxy 20(*S*) CPT (topotecan)[3,4] and 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin (irinotecan, CPT-11) demonstrated significant activity against xenografts derived from both adult and childhood malignancies.[5–13] Irinotecan is a prodrug which undergoes de-esterification by carboxylesterases to yield a much more potent topoisomerase I inhibitor, 10-hydroxy, 7-ethyl camptothecin (SN-38).

In phase I trials irinotecan has been administered by short or continuous infusion, with a variety of different schedules (reviewed in Wiseman and Markham[11]). Dose-limiting toxicity has generally

Supported in part by USPHS awards CA23099, CA32613 and CA21675 (Cancer Center Support Grant) from the National Cancer Institute, and by American, Lebanese, Syrian Associated Charities (ALSAC).

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been neutropenia and severe diarrhea.[15] The latter toxicity may be related to low glucuronidation and biliary elimination of the active metabolite SN-38.[16] Early clinical results suggest a broad spectrum of clinical activity for irinotecan as a single agent. Combination phase I trials with cisplatin,[17,18] vindesine,[19] 5-fluorouracil[20] and etoposide[21,22] have been reported. However, at this time, no evaluation of irinotecan in any childhood malignancy has been published. We recently reported the activity of i.v. administration of irinotecan against neuroblastoma xenografts.[23] In that study, significant schedule-dependent antitumor activity was demonstrated, with protracted courses of drug administration being more efficacious. In this work we have evaluated the activity of irinotecan administered by oral gavage and have defined the minimum dose associated with objective regressions in different neuroblastoma models. In addition, we have determined the daily systemic exposure to SN-38 lactone associated with objective responses in these models.

Materials and methods

Immune deprivation of mice

Female CBA/CaJ mice (Jackson Laboratories, Bar Harbor, ME) were immune-deprived as previously described.[24] Tumor pieces of approximately 3 mm^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 neuroblastoma xenografts will be described in detail elsewhere.[23] Briefly, all six neuroblastoma tumors were from young patients (1–3 years) with advanced disease. Clinical characteristics and therapy prior to being established as xenografts are presented in Table 1. With the exception of xenograft NB-EB, each tumor demonstrated amplification of N-MYC both in the biopsy and after heterografting in mice. For chemotherapy studies, all tumors were used within six passages of their engraftment in mice. Each tumor grew routinely in more than 95% of recipient mice and all were human as determined by karyotype.

Growth inhibition studies

Mice bearing bilateral s.c. tumors each received the agent when tumors were approximately 0.20–1 cm in diameter. The procedures have been reported previously.[24] 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 and mice were followed for at least 12 weeks after starting treatment.

Formulation and administration

Irinotecan powder, provided by The Pharmacia and Upjohn Company (Kalamazoo, MI), was dissolved in a solution of 0.26 ml sorbitol (70% w/w), 0.9 mg lactic acid/ml, pH 3.9 at 75°C (20 min), diluted in sterile water to 20 mg/ml, filter sterilized and stored in foil wrapped tubes (−20°C). Drug was further diluted in sterile water prior to administration (0.1 ml/20 g body weight) for either i.v. or oral routes. Irinotecan antitumor activity was evaluated on a schedule of oral daily administration for 5 days per week for 12

Table 1. Characteristics of neuroblastoma samples used for heterografting

Tumor Line	Site	Stage	Treatment[a]
NB-1382.2	retroperitoneum	C	VCR, VP-16, Cyt, CDDP, Carbo
NB-1643	retroperitoneum	D	None
NB-1691	adrenal	D	AraC, dauno, 6TG, VP-16, 5-AzaC
NB-1771	adrenal	D	None
NB-EB	adrenal	D	Cyt, Dox, CDDP, VM-26
NB-SD	bone marrow	D	Cyt, Dox, CDDP, VM-26

[a]VCR, vincristine; VP-16, etoposide; Cyt, cyclophosphamide; CDDP, cisplatin, Carbo, carboplatin; AraC, cytosine arabinoside; dauno, daunomycin; 6TG, 6-thioguanine, 5-AzaC, 5-azacytidine; VM-26, teniposide.

consecutive weeks, or for two consecutive weeks (days 1–5, 8–12; abbreviated (d × 5)2) repeated every 21 days for 4 cycles of therapy {[(d × 5)2]4} over 12 weeks. For comparison with i.v. dosing, mice received irinotecan [(d × 5)2]3, i.e. three cycles of therapy over 8 weeks, limited only by technical difficulties of further i.v. dosing. Control mice received vehicle [0.26 ml sorbitol (70% w/w), 0.9 mg lactic acid/ml, pH 3.9], diluted appropriately.

Sample collection and drug analysis

Following a single 10 mg/kg oral dose of irinotecan, blood samples were collected from mice bearing NB-1691, NB-1643 and NB-EB neuroblastoma xenografts (three animals per point) pre- and 0.25, 0.5, 1, 2 and 4 h post-administration. 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 of plasma to 800 μl of cold methanol (−30°C) followed by vigorous agitation on a vortex mixer and centrifuging again at 1250 *g* for 2 min. The supernatant was decanted and stored at −70°C until analysis.

Concentrations of irinotecan and SN-38 in plasma were determined by an isocratic HPLC assay with fluorescence detection described in detail previously.[25,26] For this study the initial wavelengths for excitation and emission were set to 375 and 500 nm, respectively. The fluorescence detector was programmed to change the emission wavelength to 540 nm (optimum emission wavelength for SN-38) 1 min prior to elution of SN-38. The lower level of quantitation was 1.0 ng/ml for both irinotecan and SN-38. All calibrators and controls were prepared in murine plasma (Hill Top Lab Animals, Scottdale, PA).

Pharmacokinetic analysis

Following oral administration of irinotecan (10 mg/kg), irinotecan and SN-38 lactone area under the plasma concentration versus time curve (AUC) were calculated by the log-trapezoidal method to the last measurable data point with extrapolation to infinity.

Elimination rate constants (k_e) for irinotecan and SN-38 were determined by log-linear regression analysis of the terminal phase of the plasma concentration–time curve. From our previous studies we have shown the disposition of irinotecan and SN-38 is linear at the dose levels evaluated in the present study.[26] Thus, AUC associated with minimum effective doses were extrapolated from the AUC calculated at the 10 mg/kg dose.

Tumor response and statistical analysis

For comparison of different treatment regimens, tumor responses were analyzed for the time (days) individual tumors required to reach four times their volume at initiation of therapy, tumor regression and the area under the estimated relative tumor volume growth curve (ARTV approach). [Relative tumor volume (RTV) = (volume day *x* after treatment/volume at initiation of treatment)]. The log linear model was used to fit relative tumor growth and the corresponding ARTV calculated. ARTVs between bilateral tumors in the same mouse appeared to be correlated; hence, for each experiment, the analyses were done based on the left-side tumor. Estimated ARTVs were compared by Wilcoxon's rank sum test.[27] The proportion of tumors that failed to reach four times their volume at the start of treatment were estimated by the Kaplan–Meier method,[28] where times to reach four times the original volume were censored in mice that died for any reason. The exact log-rank test[27,29] was used to assess differences in times for tumors to increase 4-fold in volume among treatment groups. To correct for bias from using tumors on the left flank only, the same analyses were done on tumors randomly selected from both sides for each mouse. For individual tumors partial response (50% or more volume regression; PR) was defined as a volume regression greater than 50% but with measurable tumor at all times. Complete response (CR) was defined as disappearance of measurable tumor mass at some point after initiating therapy. Maintained complete response (MCR) was CR without tumor regrowth within the study time frame (12 weeks). For estimating the minimum irinotecan dose associated with objective responses for treatment groups required that all tumors within that group regressed 50% or more.

Results

Toxicity and efficacy of protracted oral administration (d × 5)12

Results with neuroblastoma xenografts, and other xenograft models examined,[23,11] indicated that extending the duration of irinotecan therapy was more efficacious than increasing dose intensity. Conse-

quently, as protracted administration could be more easily accomplished by dosing via the oral route (p.o.), we examined the efficacy of irinotecan given on a (d × 5) schedule for 12 consecutive weeks. The MTD was 50 mg/kg/dose although lethality was about 4%. Treatment at the MTD was associated with loose feces starting at week 8, a high incidence of eye infections associated with *Moraxella* (an organism associated with the conjunctival flora of CBA/CaJ mice) and broken teeth. Mice lost weight during the course of treatment, weighing 81 ± 4% (SD; $n = 6$ experiments) at the end of treatment and failed to gain weight during the next month of observation. However, given at 50 or 25 mg/kg/dose on this schedule, irinotecan was highly active, causing CR of all tumors (Table 2). Representative experiments are shown in Figure 1 for NB-1771 tumors.

Toxicity and efficacy of protracted intermittent schedules of oral irinotecan

Because of the pronounced and poorly reversible weight loss associated with the (d × 5)12 schedule of administration, we evaluated an intermittent schedule of dosing, similar to that we have used for i.v. studies.[11] Mice received irinotecan for 5 days on two consecutive weeks (one course) with courses repeated every 21 days for four cycles over 12 weeks. The MTD for this schedule was 75 mg/kg/dose and overall was tolerated well with no deaths, and body weight was 92 ± 4% (SD; $n = 6$ experiments) at the end of treatment. Thus, for the same total dose administered as the continuous schedule (3 g/kg), the intermittent schedule allowed for recovery of body weight between cycles of administration and was better tolerated. Both 75 and 50 mg/kg/dose caused CR of all neuroblastoma xenografts examined (data not shown). However, we have shown previously that mice tolerate much higher systemic exposure to irinotecan and its active metabolite SN-38, than can be tolerated by patients at the respective MTDs.[30] We were therefore interested in determining the lowest dose levels, using the [(d × 5)2]4 schedule that caused objective regressions in these neuroblastoma models. Results are presented in Table 3. Objective regressions were obtained in NB-SD and NB-1691 xenografts at a dose of 12.5 mg/kg, and in NB-1643 at 6.25 mg/kg. The

Table 2. Responses of neuroblastoma xenografts to oral irinotecan (d × 5) 12 schedule[a]

Tumor	Dose (mg/kg schedule)	Days to 4 times (± SE)[b]	GD[c]	PR (%)	CR (%)	MCR (%)
NB-1691	0	16.9 ± 1.7				
	25 (d × 5) 12 p.o.	> 84	67.1	0	100	100
	50 (d × 5) 12 p.o.	> 84	67.1	0	100	100
NB-1643	0	28.0 ± 0.3				
	25 (d × 5) 12 p.o.	> 84	56	0	100	100
	50 (d × 5) 12 p.o.	> 84	56	0	100	100
NB-1382.2	0	24.5 ± 0.8				
	25 (d × 5) 12 p.o.	> 84	59.5	0	100	100
	50 (d × 5) 12 p.o.	> 84	59.5	0	100	100
NB-1771	0	39.5 ± 1.8				
	25 (d × 5) 12 p.o.	> 84	44.5	0	100	100
	50 (d × 5) 12 p.o.	> 84	44.5	0	100	100
NB-SD	0	20.3 ± 1.6				
	25 (d × 5) 12 p.o.	> 84	63.7	0	100	100
	50 (d × 5) 12 p.o.	> 84	63.7	0	100	100
NB-EB	0	10.5 ± 0.7				
	25 (d × 5) 12 p.o.	> 84	73.5	0	100	100
	50 (d × 5) 12 p.o.	> 84	73.5	0	100	100

[a]PR, 50% or greater regression; CR, complete regression; MCR, maintained CR at 12 weeks.
[b]Days to 4 times: days for tumors to grow to four times their volume at the initiation of therapy.
[c]Growth delay (time to 4 times for treated tumors − time to 4 times for control tumors).

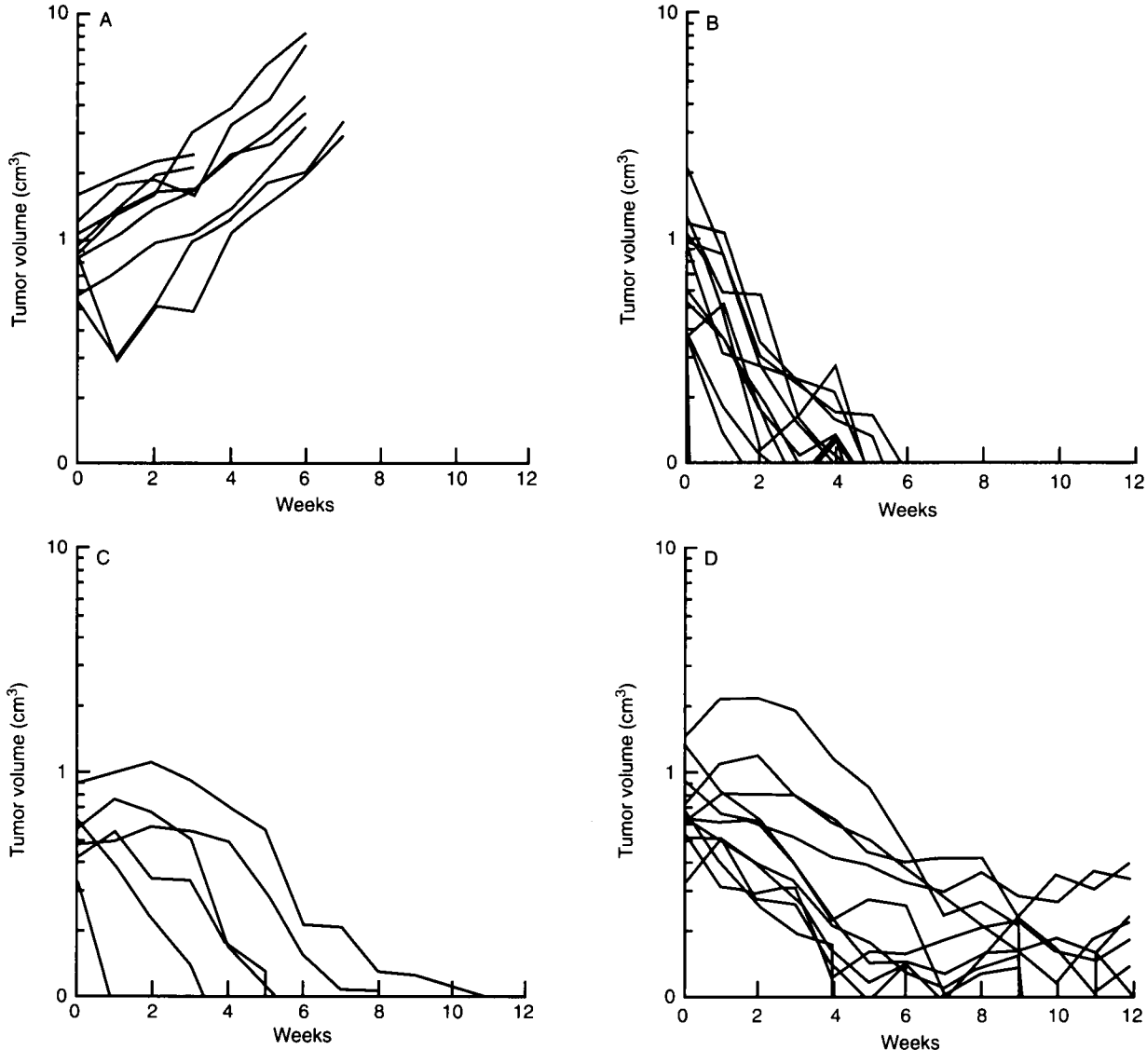

Figure 1. Responses of NB-1771 neuroblastoma xenografts to oral irinotecan. Treatment was started when tumor volumes were 0.2 cm^3 or greater. Mice received oral administration (d × 5) for 12 consecutive weeks. (A) Controls, (B) 50, (C) 12.5 and (D) 6.25 mg/kg. Each curve shows the growth of an individual tumor.

lowest dose examined in NB-EB xenografts (12.5 mg/kg) caused CR maintained at week 12. Representative results obtained with NB-SD xenografts are shown in Figure 2. Irinotecan significantly inhibited growth of NB-SD tumors at dose levels as low as 6.25 mg/kg ($p < 0.0041$). Thus, our data suggest that for a majority of neuroblastoma models evaluated, oral dose levels of 6.25–12.5 mg/kg are associated with objective tumor regressions. For comparison between p.o. and i.v. efficacy, data for NB-SD tumors for i.v. therapy with irinotecan given at 20% of the p.o. dose (to adjust for oral bioavailability) on a similar schedule is shown in Figure 3.

Irinotecan and SN-38 systemic exposures associated with objective tumor regressions

The daily systemic exposures (AUC) of SN-38 lactone associated with the minimum dose achieving objective responses in NB-1691 (12.5 mg/kg), NB-1643 (6.25 mg/kg) and NB-EB (12.5 mg/kg) for SN-38 lactone were 704, 418 and 987 ng-h/ml, respectively. In addition, the daily AUC of irinotecan lactone associated with the minimum dose achieving response were 219, 152 and 653 ng-h/ml, respectively.

Table 3. Responses of neuroblastomas to intermittent scheduled oral irinotecan[a]

Tumor	Dose (mg/kg schedule)	Days to 4 times (± SE)[b]	GD[c]	PR (%)	CR (%)	MCR (%)
NB-1691	0	10.5 ± 1.5				
	12.5 [(d × 5)2]4	81	71	15	85	69
	6.25 [(d × 5)2]4	40.4	30	55	9	0
	3.1 [(d × 5)2]4	46.9	36	10	30	0
NB-1643	0	20.7 ± 1.6				
	6.25 [(d × 5)2]4	> 84	63	30	70	50
	3.1 [(d × 5)2]4	75.9	55	54	23	15
NB-1771	0	35.0 ± 2.0				
	6.25 [(d × 5)2]4	83	48	17	25	17
	3.1 [(d × 5)2]4	40	5	0	11.1	11
NB-SD	0	17.0 ± 1.3				
	25 [(d × 5)2]4	> 84	68	8	92	92
	12.5 [(d × 5)2]4	> 84	68	39	62	46
	6.25 [(d × 5)2]4	34	18	0	0	0
	3.1 [(d × 5)2]4	20	3	0	0	0
NB-ED	0	13.0 ± 1.6				
	25 [(d × 5)2]4	> 84	71	0	100	100
	12.5 [(d × 5)2]4	> 84	28	0	100	100

[a]See footnotes to Table 2.

Discussion

Although oral administration of irinotecan has been reported to demonstrate efficacy in rodent models,[5,31] this is the first report of the systemic exposure of irinotecan and SN-38 lactone associated with antitumor activity in mice following oral administration. The purpose of this study was to evaluate the efficacy of a new antitumor agent, irinotecan, after oral administration against a panel of neuroblastoma xenografts. Limited studies have been reported that demonstrate activity of irinotecan in neuroblastoma xenografts.[12,13] In our study six neuroblastoma xenografts from patients with stage C or D disease were examined for sensitivity to irinotecan. Each xenograft, except NB-EB, exhibited N-MYC amplification, thus representing tumors that clinically have an extremely poor prognosis.[32,33]

We have suggested that pulsing drugs such as irinotecan by daily bolus administration over a prolonged period of time may prove efficacious, especially in tumors that had only a small proportion of S phase cells. Consistent with this, our studies[10,11] have demonstrated that low doses of irinotecan given for extended periods were more efficacious than more intensive courses of therapy against several types of human tumor xenografts. As protracted schedules would be more acceptable if irinotecan could be administered orally, we have evaluated this route of administration using two schedules. Given for 5 days per week for 12 consecutive weeks, the MTD was 50 mg/kg/dose p.o. Complete regression of all neuroblastoma lines was achieved at the MTD and at 25 mg/kg/dose. However, toxicity associated with the MTD was significant, manifest by both persistent weight loss, eye infections and broken teeth. Data are accumulating to suggest mice may tolerate substantially greater systemic exposure to irinotecan and its active metabolite SN-38 at the respective MTD, than can be achieved in patients[26] (unpublished data). We therefore lowered the irinotecan dose to define the minimum daily dose that resulted in objective regressions in these tumor models. For these studies an intermittent schedule of administration [(d × 5)2]4 was used to more closely simulate previous i.v. scheduling.[11,23] For most of the neuroblastoma xenografts evaluated, objective regressions were observed at doses of 6.25 and 12.5 mg/kg/day.

The clinical significance of evaluating optimal irinotecan schedules and routes of administration in xenograft models lies in the ability to achieve similar systemic exposures in patients that are associated with antitumor response in the xenograft model. In addition, comparing systemic exposures after oral and i.v. administration provides a method to compare efficacy between alternative routes of administration. When comparing the systemic exposures associated with the minimum dose achieving tumor response after oral and i.v. irinotecan administration,

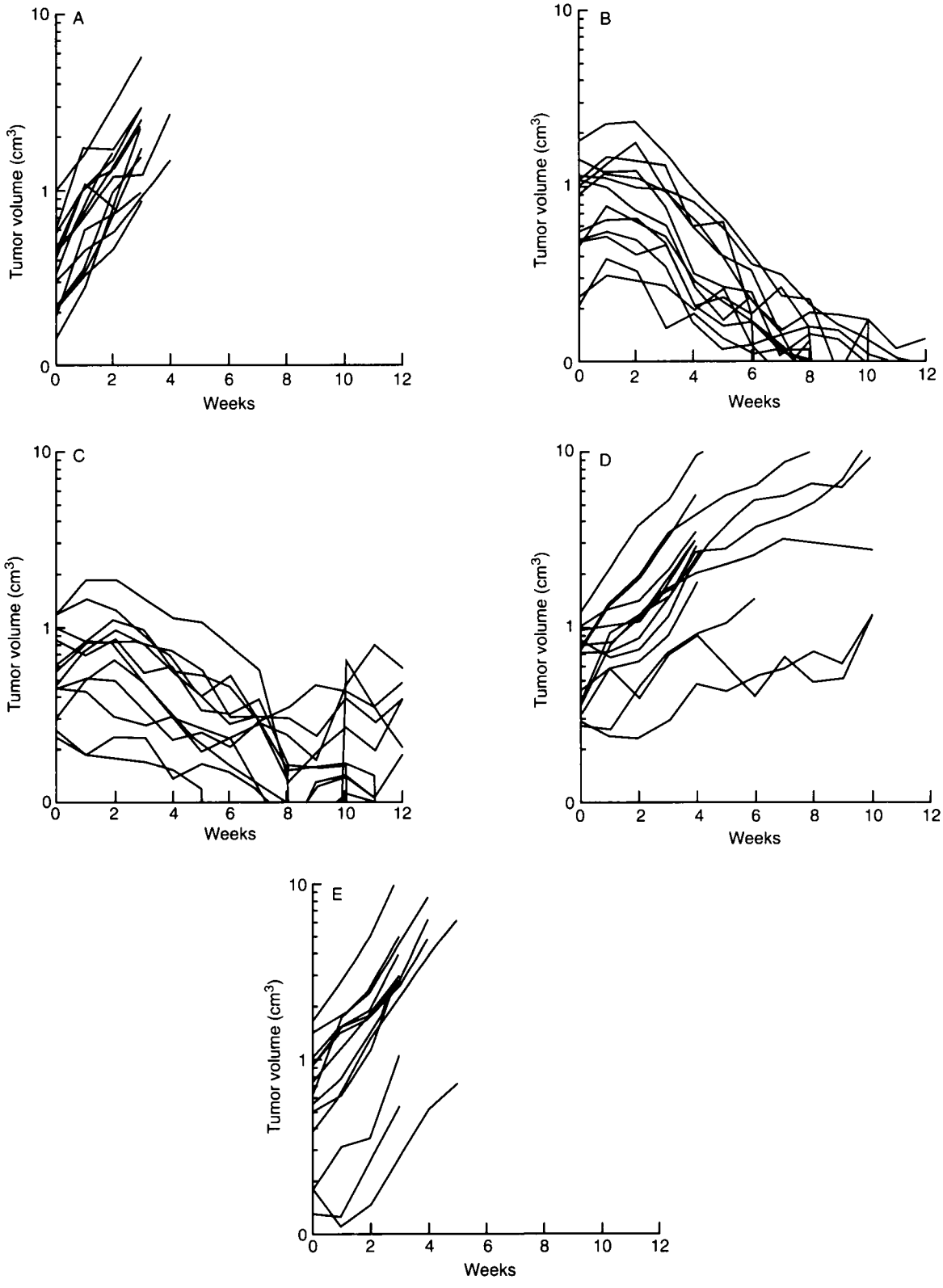

Figure 2. Responses of NB-SD neuroblastoma xenografts to an intermittent schedule of oral irinotecan. Treatment was started when tumor volumes were 0.2 cm^3 or greater. Mice received p.o. administration on a (d × 5)2 schedule repeated every 21 days for four cycles of therapy. (A) Control, (B) 25, (C) 12.5, (D) 6.25 and (E) 3.1 mg/kg. Each curve shows the growth of an individual tumor.

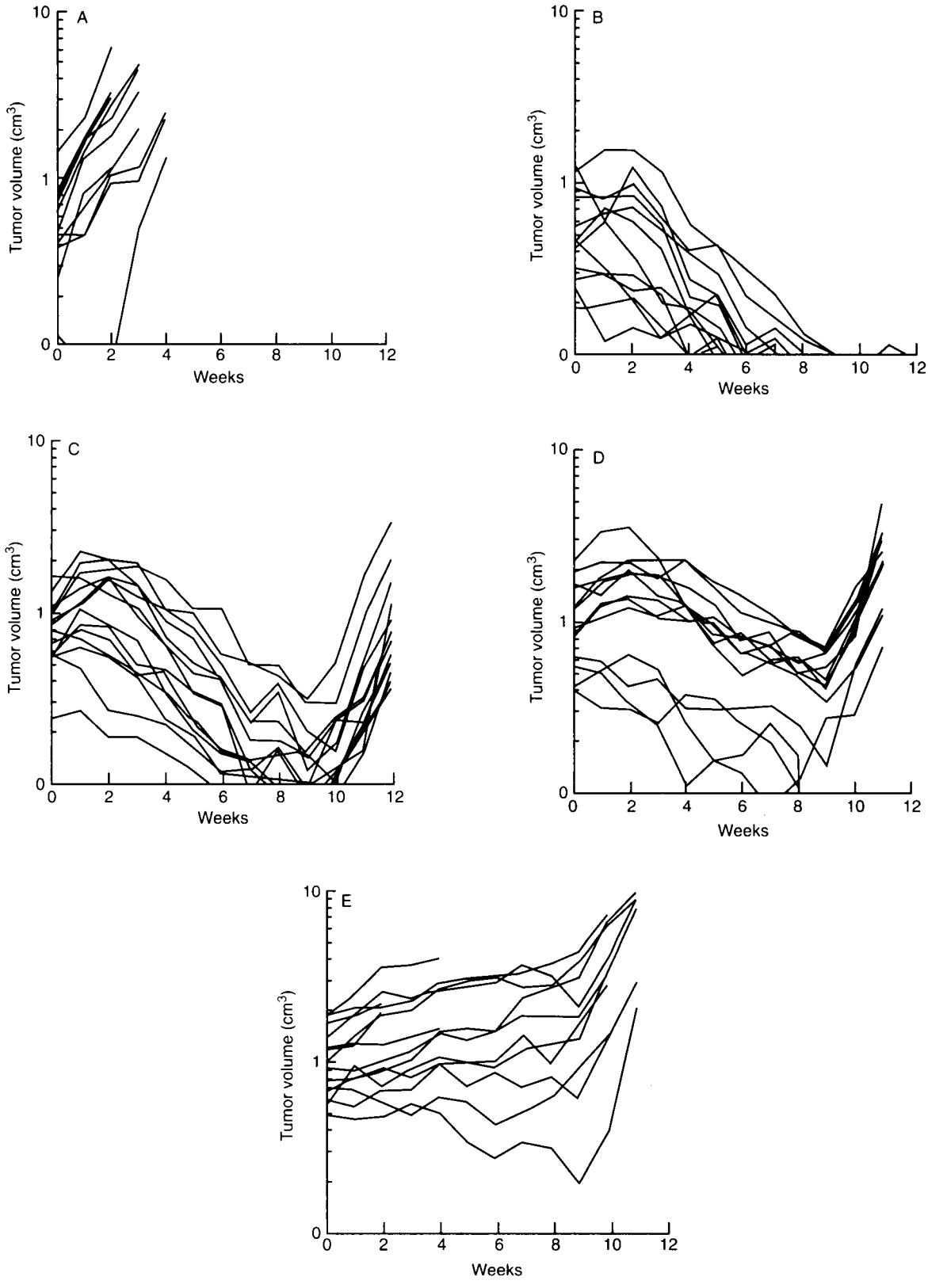

Figure 3. Responses of ND-SD neuroblastoma xenografts to an intermittent schedule of i.v. irinotecan. Irinotecan was administered i.v. on the [(d × 5)2]3 schedule when tumors were 0.2 cm^3 or greater. (A) Controls, (B) 5, (C) 2.5, (D) 1.25 and (E) 0.61 mg/kg. Each curve shows the growth of an individual tumor.

there are significant differences in SN-38 systemic exposure. After i.v. irinotecan administration objective responses were obtained at 1.25 mg/kg (NB-1691, NB-1643 and NB-EB), daily SN-38 lactone systemic exposures associated with response in NB1691, NB1643 and NBEB were 129, 99 and 114 ng-h/ml, respectively.[23] These SN-38 systemic exposures are 5.5-, 4.2- and 8.7-fold lower than required to achieve similar tumor regressions after oral irinotecan. We are currently investigating the potential mechanisms responsible for these observations. Interestingly, the irinotecan systemic exposures causing similar tumor responses after oral and i.v. administration are similar for NB-1691 (291 and 220 ng-h/ml, respectively) and NB-1643 (152 and 198 ng-h/ml, respectively). Whereas for the NB-EB xenograft the irinotecan AUCs are significantly different being 653 and 175 ng-h/ml, respectively. Regardless of route of administration, equal systemic exposures should, hypothetically, achieve similar antitumor response when the profile and duration of exposure are similar. In the case of oral and i.v. administration of irinotecan, results for SN-38 are at variance with this hypothesis, whereas data for irinotecan appears to relate more closely with antitumor activity. Thus, these data suggest irinotecan systemic exposure may better predict tumor response than SN-38 systemic exposure after oral administration in some xenograft tumor lines. Further studies are necessary to either confirm or refute this conjecture.

Conclusion

In summary, the sensitivity of childhood neuroblastoma to oral administration of irinotecan has been established. Results indicate that neuroblastoma xenografts are sensitive to oral irinotecan and that protracted schedules are equally efficacious as i.v. administration. Estimation of the lowest effective dose using the protracted p.o. schedule suggests that neuroblastoma xenografts may respond to daily doses as low as 6.25–12.5 kg/kg (about 20–40 mg/m^2). Thus objective tumor responses can be achieved at dose levels representing 8% of the MTD for p.o. administration. For i.v. administration, we have reported objective regressions in neuroblastoma models at 1.25 mg/kg/dose on the intermittent schedule reported here. This represents about 3% of the MTD. Thus for both p.o. and i.v. administration, irinotecan demonstrates a high therapeutic index, based largely on the tolerance of mice for this class of antitumor agent. Further, the secretory diarrhea, dose limiting in many clinical trials, is not recapitulated in the mouse model. Thus, there is potential to increase this toxicity by oral dosing that cannot be predicted from the model. An initial report[34] suggests that toxicity from oral dosing in humans is similar to that observed after systemic administration of irinotecan. In our study, objective responses after p.o. administration were associated with systemic exposures of SN-38 ranging from 417 to 987 ng-h/ml and irinotecan ranging from 152 to 653 ng-h/ml. Moreover, our data suggest in order to optimize response after oral administration of irinotecan in patients with neuroblastoma similar SN-38 and or irinotecan systemic exposures should be achieved as those associated with antitumor response in the xenograft model.

Acknowledgments

We are grateful to J Patrick McGovren for supplying irinotecan for these studies and Susan Cohn for facilitating access to tumor material through the Pediatric Oncology Group.

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(Received 9 January 1997; accepted 30 January 1997)