Cancer Chemother Pharmacol (1995) 36: 45–52

ORIGINAL ARTICLE

Peter J. Houghton · Pamela J. Cheshire
James D. Hallman II · Janet A. Houghton

Therapeutic efficacy of the cyclopropylpyrroloindole, carzelesin, against xenografts derived from adult and childhood solid tumors

Received: 2 May 1994/Accepted: 22 September 1994

Abstract The therapeutic efficacy of the sequence-selective, DNA minor-groove-binding alkylating agent carzelesin was evaluated against a series of human tumor xenografts growing at the s.c. site. The model consisted of seven colon adenocarcinomas, and six pediatric rhabdomyosarcomas. In addition, carzelesin was evaluated against xenografts selected in situ for resistance to vincristine, melphalan, and topotecan. Carzelesin was given as a single i.v injection, and tumor volumes were determined at 7-day intervals. At the highest dose [0.5 mg/kg, the dose producing 10% lethality (LD_{10})]), carzelesin significantly inhibited growth in four of six colon tumor lines, causing a high proportion of partial regressions in one of seven lines and complete regressions of VRC_5 colon tumors. At 0.25 mg/kg, significant growth inhibition was determined in only two of seven colon tumor lines with infrequent volume regressions. Carzelesin given at the highest nonlethal dose level significantly inhibited the growth of each of six rhabdomyosarcomas, causing a high frequency of partial or complete regressions in four of six tumor lines. There was no apparent cross-resistance to carzelesin in two rhabdomyosarcomas selected for vincristine resistance (Rh12/VCR, Rh18/VCR) or in Rh28/LPAM xenografts selected for primary resistance to the bifunctional alkylating agent melphalan. Interestingly, carzelesin maintained full activity against Rh18/TOPO tumors selected in situ for resistance to topotecan, whereas the colon tumor VRC_5/TOPO, selected in a similar manner, was completely resistant to this agent.

Key words Carzelesin · Human tumor xenrografts · Drug resistance · Adenocarcinoma · Rhabdomyosarcoma

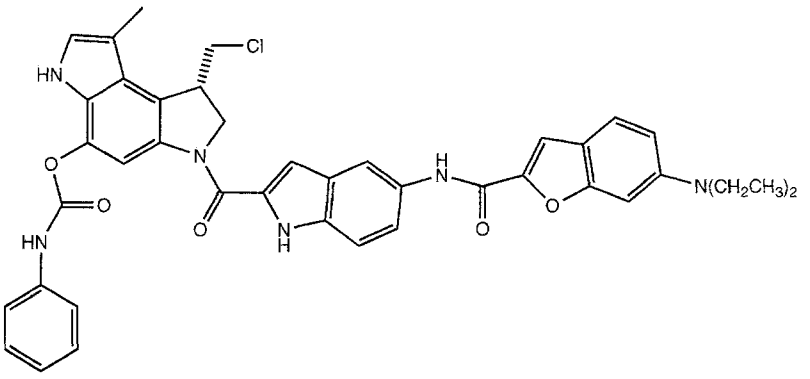
Introduction

Carzelesin (Fig. 1) is one of three semisynthetic cyclopropylpyrroloindole (CPI) antitumor agents to enter phase I clinical trials [13]. These analogues are DNA minor-groove-binding, sequence-selective alkylating agents modeled on the cytotoxic antibiotic CC-1065. CPI analogues contain a cyclopropyl group that mediates the formation of N^3-adenine covalent adducts in double-stranded DNA. Adozelesin was selected for clinical development on the basis of its broad-spectrum activity against mouse tumors and human xenografts, its high potency, and its pharmaceutical properties [14]. Subsequently, synthetic efforts were directed at the preparation of CPI prodrugs, resulting in the identification of carzelesin as an agent with superior therapeutic activity over adozelesin [13–15]. Carzelesin demonstrated increased efficacy against L1210 leukemias and several rodent solid tumors as well as a panel of early-stage human xenografts, including colon (CX-1), lung (LX-1), ovarian 2780, and prostate DU-145. Further testing showed that carzelesin caused marked regressions of advanced-stage ovarian 2780 tumors [13]. In this study, the efficacy of carzelesin was examined against an expanded panel of tumors derived from colon adenocarcinomas and childhood rhabd omyosarcomas. Adenocarcinomas of the colon are generally refractory to alkylating agents, whereas childhood rhabdomyosarcomas are often exquisitely sensitive to bifunctional alkylating agents such as cyclophosphamide, ifosfamide, and melphalan [5–8, 10].

Supported in part by grants CA23099 and CA21675 (Cancer Center Grant) by American Lebanese Syrian Associated Charities (ALSAC).

P.J. Houghton (✉) · P.J. Cheshire · J.D. Hallman II · J.A. Houghton
Laboratories for Experimental Therapeutics, Department of Molecular Pharmacology, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis TN 38101, USA

Fig. 1 Chemical structure of carzelesin



Materials and methods

Immune deprivation of mice

Female CBA/CaJ mice (Jackson Lab, Bar Harbor, Me) aged 4 weeks 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 of irradiation [9].

Tumor lines

Each of the independently derived lines from previously untreated rhabdomyosarcomas (RMS) and colon adenocarcinomas have been described elsewhere [3]. For chemotherapy studies, all tumors were used within 32 passages of their engraftment in mice. Each tumor grew routinely in over 90% of recipient mice, and all tumors were 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 [5], for melphalan (L-PAM) [6], and for topoisomerase I inhibitors [2, 4].

Sublines of Rh12, Rh18 and Rh28 selected in situ for resistance to vincristine (Rh12/VCR-3, Rh18/VCR-3) melphalan, (Rh28/L-PAM), and topotecan (Rh18/TOPO) have been described elsewhere [3]. The colon adenocarcinomas used (HC_1, GC_3, VRC_5 and ELC_2) were derived from adult patients and have been characterized extensively [3,8,11]. SJC_2 is a moderately differentiated adenocarcinoma isolated from a 14-year-old girl, SJC_3A and SJC_3B tumors were independent primaries in a 26-year-old man, and SJC_8 is a well-differentiated adenocarcinoma isolated from an 11-year-old boy.

Growth-inhibition studies

Mice bearing bilateral s.c. tumors received the agent when tumors were approximately 0.4–1 cm in diameter. Tumor response was determined at 7-day intervals using digital calipers (Maxcal) interfaced to a Dell 486/50 microcomputer. Two perpendicular diameters were used to compute volumes [3]. Growth delay was calculated from the difference in days required for treated tumors to grow to 4-fold their volume at the time of treatment initiation as compared with vehicle-treated controls. For each treatment group, six or seven tumor-bearing mice were used. Relative tumor volumes were calculated from the formula $RTV = (V_x/V_0)$, where V_x is the volume on day X and V_0 is the volume of tumor at time of treatment initiation. Grading of tumor responses is given in Table 1; the definition of $\geq 50\%$ regression required that at some time point after treatment, each tumor within a treatment group demonstrate such a reduction in volume. To equate responses in tumor lines that demonstrate different rates of growth, inhibition was normalized by expressing this as a function of tumor volume-doubling time (see Tables 4, 5).

Formulation and administration

Stock solutions of carzelesin were prepared in *N,N*-dimethylacetamide (DMA) and stored in foil-wrapped containers at − 20°C for up to 7 days. For administration, stock solutions were diluted in sterile water to 12.5–50 μg/ml containing a final concentration of 2% DMA and 10% emulphor. Carzelesin was given as a single i.v. injection (0.1 ml/10 g body weight). Carzelesin was generously supplied by Dr. J. Patrick McGovren (The Upjohn Company, Kalamazoo, Mich.).

Statistical analysis

The results of individual tumor-inhibition studies were analyzed with one-way analysis of variance using the number of days required for tumors to reach four times their original 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 its original volume was assigned a default value of the maximal duration of the study. For comparison of the efficacy of various courses of treatment, data were collapsed across studies, within a tumor line. The percentage of tumors showing partial and/or complete regression and any regrowth were calculated for the individual tumor lines as previously described [3].

Results

Toxicity

Given as a single administration i.v, the maximum tolerated dose of carzelesin was 0.5 mg/kg. This dose level resulted in 14 deaths among the 201 mice treated. Of these, 12 mice were bearing either Rh30 ($n = 7$) or IRS 56 ($n = 5$) xenografts, both of which were highly volume-responsive to treatment. Consequently, it was not possible to determine whether toxicity in these mice was secondary to rapid tumor regression rather than being directly related to carzelesin toxicity. However, 0.5 mg/kg probably represents an approximation of the dose producing 10% lethality (LD_{10}), if all deaths are

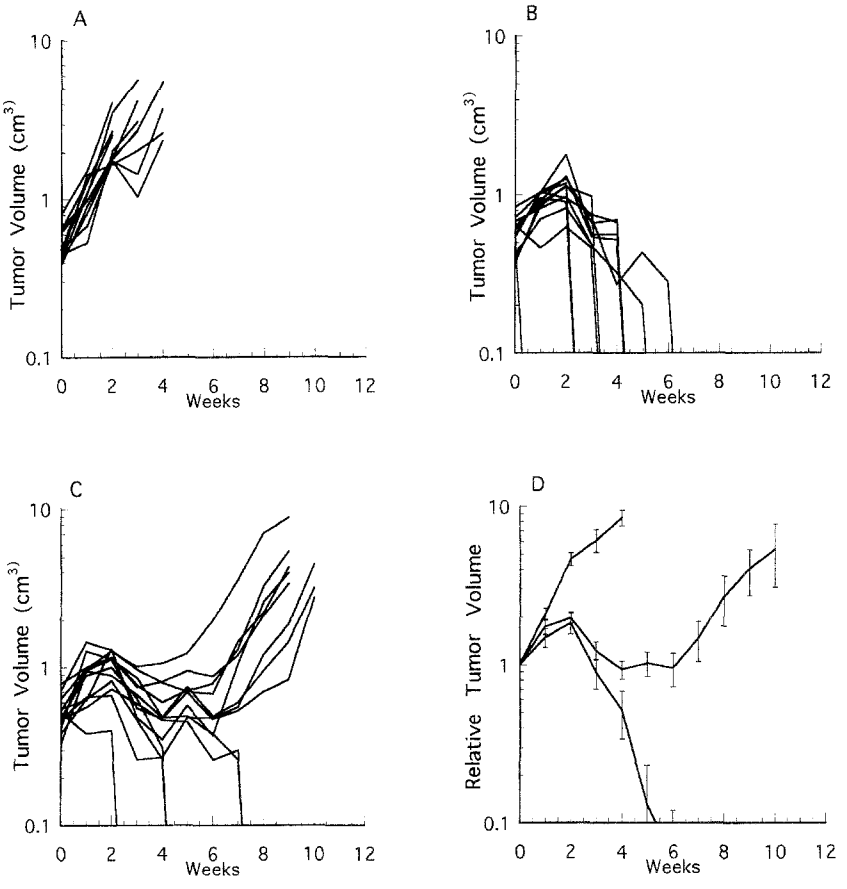


Fig. 2A–D Responses of VRC_5 colon adenocarcinoma xenografts to carzelesin. Mice received **A** vehicle or carzelesin at **B** 0.5 or **C** 0.25 mg/kg i.v as a single administration. **A–C** Growth patterns of individual tumors. **D** Mean growth curves generated for each treatment group ± SE

included. In addition, mice bearing the SJC_3B colon xenograft did not tolerate dose levels greater than 0.25 mg/kg, although tumors were not volume-responsive at lower dose levels.

Colon carcinomas

Carzelesin given at the optimal dose was most active against VRC_5 xenografts, causing a high frequency of complete regressions (21/22 tumors in replicate experiments), and also caused partial and complete responses at 0.25 mg/kg, (Fig. 2). In contrast, the other six colon tumor lines were relatively unresponsive. Although carzelesin caused significant growth stasis in two additional lines (GC_3 and ELC_2), there were few partial regressions except in SJC_3A xenografts. Data are summarized in Tables 1 and 4.

Rhabdomyosarcomas

When the same doses and schedule of administration were used, carzelesin caused significant regressions of advanced Rh18, IRS 56, and IRS68 RMS xenografts. Responses of Rh18 tumors are shown for a representative experiment in Fig. 3. Data for representative RMS studies are summarized in Table 2. Analysis of pooled data from several repeated studies with several lines of RMS xenografts showed a high proportion of partial (PR) and complete (CR) regressions. Rh18, 19 PR + 17 CR ($n = 42$); Rh30, 5 PR + 34 CR ($n = 44$); IRS68, 17 PR + 13 CR ($n = 34$); each of 18 IRS56 tumors regressed completely. However, reduction of the delivered dose of carzelesin resulted in a marked decrease in objective responses in all tumors except Rh30 and IRS56, (Tables 2, 5).

Cross-resistance to carzelesin

Carzelesin was evaluated in two rhabdomyosarcomas selected in vivo for resistance to vincristine (Rh12/VCR and Rh18/VCR) and against a subline of Rh28 selected for primary resistance to L-PAM. Rh28/LPAM is cross-resistant to vincristine and also to VP-16 [1,7]. Carzelesin had similar activity against vincristine resistant sublines and parental RMSs, (Table 3). Rh28 and its melphalan-resistant subline had similar sensitivity, although parental Rh28 tumors were only marginally sensitive to carzelesin. Responses to carzelesin of two

Table 1 Sensitivity of human colon adenocarcinoma xenografts to carzelesin

Tumor	Dose (mg/kg)[a]	Time to 4X (days)	Growth delay (days)	T/C[b] (day)	Response (%)[c] PR	CR	C	(*n*)
HC_1	0	41.3 ± 17						
	0.5	51.0 ± 17	9.7	0.68(35)	7	0	0	(14)
GC_3	0	27.9 ± 12						
	0.125	41.0 ± 17	13.1	0.71 (21)	0	0	0	(14)
	0.25	52.3 ± 17	24.4	0.53 (21)	0	0	8	(12)
	0.5	59.3 ± 15	31.4*	0.35 (21)	8	0	0	(12)
VRC_5	0	14.1 ± 4						
	0.125	82.0 ± 5	67.9*	0.1 (28)	50	35	35	(14)
	0.25	68.0 ± 14	53.9*	0.1 (28)	0	31	31	(13)
	0.5	> 84	> 70*	0.06 (28)	0	100	100	(12)
ELC_2	0	33.8 ± 9						
	0.25	83.3 ± 3	49.5*	0.21 (35)	0	0	0	(14)
	0.5	> 84	> 50*	0.17 (28)	7	0	0	(14)
SJC_3A	0	17.9 ± 8.9						
	0.25	38.1 ± 8.7	20.2	0.26 (14)	20	0	0	(10)
	0.5	55.0 ± 5.5	37.1*	0.17 (14)	70	0	0	(10)
SJC_3B	0	25.9 ± 13						
	0.125	33.5 ± 7	7.6	0.57 (14)	0	0	0	(12)
	0.25	44.5 ± 6	18.6	0.35 (14)	0	0	0	(12)
	0.5	Toxic	–	–	–	–	–	–
SJC_8	0	22.9 ± 5						
	0.25	37.6 ± 20	14.7	0.81 (14)	0	0	0	(14)
	0.5	33.1 ± 7	10.2	0.64 (21)	0	0	0	(14)

* Significantly different from control ($P < 0.05$)
[a] Carzelesin given as a single administration i.v.
[b] T/C: maximal treated/control volume ratio; day of measurement given in parentheses
[c] PR, partial response (≥ 50% volume regression); CR, complete response; C, no regrowth during period of observation (84 days)

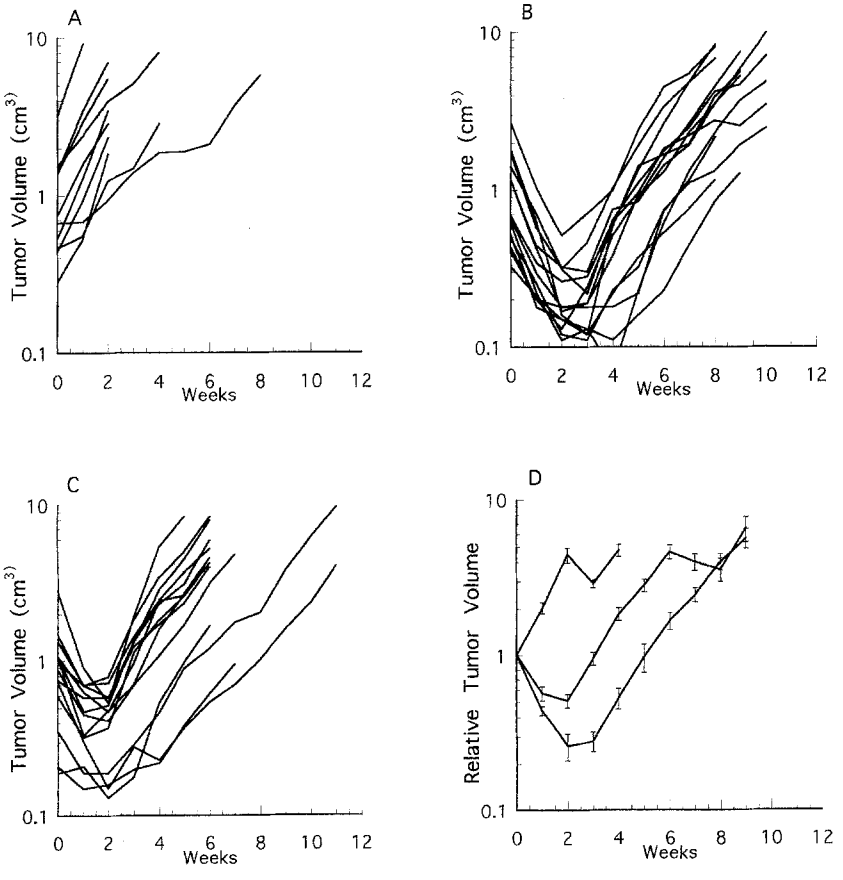


Fig. 3A–D Responses of Rh18 rhabdomyosarcoma xenografts to carzelesin. Mice received **A** vehicle or carzelesin at **B** 0.5 or **C** 0.25 mg/kg i.v as a single administration. **A–C** Growth patterns of individual tumors. **D** Mean growth curves generated for each treatment group ± SE

Table 2 Sensitivity of childhood rhabomyosarcomas to carzelesin

Tumor	Dose (mg/kg)[a]	Time to 4X (days)	Growth delay (days)	T/C[b] (day)	Response (%)[c] PR	CR	C	(n)
Rh12	0	27.6 ± 14						
	0.25	58.5 ± 9	30.9*	0.22 (21)	0	0	0	(14)
	0.5	70.2 ± 10	42.6*	0.21 (35)	11	0	0	(9)
Rh18	0	12.2 ± 4						
	0.125	22.9 ± 7	10.7	0.30 (14)	0	0	0	(14)
	0.25	32.5 ± 9	20.3	0.25 (14)	14	7	0	(14)
	0.5	67.7 ± 15	55.5*	0.07 (21)	14	50	28	(14)
Rh28	0	26.8 ± 15						
	0.25	33.4 ± 12	6.4	0.52 (14)	0	0	0	(11)
	0.5	46.2 ± 14	19.4	0.23 (14)	26	8	0	(13)
Rh30	0	6.2 ± 4						
	0.125	48.9 ± 20	42.7*	0.09 (7)	54	31	8	(13)
	0.25	77.2 ± 11	71.0*	0.18 (7)	0	100	46	(11)
	0.5	> 84	> 78*	0.13 (7)	0	100	100	(12)
IRS56	0	32.4 ± 9						
	0.25	81.1 ± 6	48.7*	0.06 (6)	0	100	81	(11)
	0.5	> 84	> 52*	0.0 (6)	0	100	100	(14)
IRS68	0	26.0 ± 14						
	0.25	40.1 ± 12	14.1	0.20 (21)	24	0	0	(12)
	0.5	72.4 ± 10	46.4*	0.06 (21)	92	8	0	(13)

* Significantly different from control ($P < 0.05$)
[a] Carzelesin given as a single administration i.v.
[b] T/C: maximal treated/control volume ratio; day of measurement given in parentheses
[c] PR, partial response (≥ 50% volume regression); CR, complete response; C, no regrowth during period of observation (84 days)

Table 3 Sensitivity of carzelesin of colon and rhabomyosarcomas selected in situ for resistance to chemotherapeutic agents

Tumor	Dose (mg/kg)[a]	Time to 4X (days)	Growth delay (days)	T/C[b] (day)	Response (%)[c] PR	CR	C	(n)
Rh18/TOPO	0	14.6 ± 10						
	0.25	50.9 ± 24	36.3*	0.13 (14)	67	0	0	(12)
	0.5	80.6 ± 7	66.0*	0.03 (14)	21	79	0	(14)
Rh18/VCR	0	27.1 ± 12						
	0.25	78.3 ± 10	51.2*	0.05 (28)	70	30	30	(10)
	0.5	81.8 ± 5	54.7*	0.01 (35)	24	76	61	(13)
Rh12/VCR	0	22.7 ± 5						
	0.5	62.1 ± 18	39.4*	0.12 (28)	86	0	0	(14)
Rh28/LPAM	0	13.6 ± 8						
	0.25	20.7 ± 7	7.1	0.46 (14)	0	0	0	(11)
	0.5	30.2 ± 5	16.6	0.25 (14)	7	0	0	(14)
VRC_5/TOPO	0	9.6 ± 4						
	0.25	14.1 ± 3	4.5	0.44 (14)	0	0	0	(12)
	0.5	16.9 ± 10	7.3	0.28 (14)	0	0	0	(12)

* Significantly different from control ($P < 0.05$)
[a] Carzelesin given as a single administration i.v.
[b] T/C: maximal treated/control volume ratio; day of measurement given in parentheses
[c] PR, partial response (≥ 50% volume regression); CR, complete response; C, no regrowth during period of observation (84 days)

topotecan-resistant tumors are presented in Fig. 4 and 5. In contrast to the parental line, the VRC_5/TOPO colon carcinoma was unresponsive to carzelesin (Fig. 4), whereas Rh18/TOPO retained sensitivity similar to that of its parental xenograft (Fig. 5).

Discussion

Carzelesin is one of three cyclopropylpyrroloindole (CPI) analogues to enter phase I clinical trial. These are

Table 4 Acitivity of carzelesin against human colon adenocarcinoma xenografts

Tumor	Dose	Response[a]
HC_1	0.5	–
GC_3	0.125	–
	0.25	±
	0.50	+ +
VRC_5	0.125	+ + +
	0.25	+ + +
	0.50	+ + + + + +
ELC_2	0.25	+ +
	0.5	+ + +
SJC3A	0.25	–
	0.5	+ +
SJC3B	0.125	–
	0.25	+
SJC8	0.25	–
	0.5	–

[a] –, No growth inhibition; +, growth inhibition of ≥ 1 tumor volume – doubling time (Td_2); + +, inhibition of $\geq 2 \times Td_2$; + + +, inhibition of $\geq 3 \times Td_2$; + + + +, inhibition of $\geq 3 \times Td_2$ + 50% regression of all tumors; + + + + +, complete regression of all tumors; + + + + + +, CR of all tumors without regrowth during period of observation

Table 5 Acitivity of carzelesin against rhabdomyosarcoma xenografts

Tumor	Dose	Response[a]
Rh12	0.25	+ +
	0.5	+ + +
Rh18	0.125	+
	0.25	+ + +
	0.5	+ + + +
Rh28	0.25	–
	0.5	+
Rh30	0.125	+ + +
	0.25	+ + + + +
	0.5	+ + + + + +
IRS56	0.25	+ + + + +
	0.5	+ + + + + +
IRS68	0.125	–
	0.25	–
	0.5	+ + +

[a] –, No growth inhibition; +, growth inhibition of ≥ 1 tumor volume – doubling time (Td_2); + +, inhibition of $\geq 2 \times Td_2$; + + +, inhibition of $\geq 3 \times Td_2$; + + + +, inhibition of $\geq 3 \times Td_2$ + 50% regression of all tumors; + + + + +, complete regression of all tumors; + + + + + +, CR of all tumors without regrowth during period of observation

highly potent, sequence-selective DNA-binding agents. Clinical development was based upon several criteria, one of which was significant broad-spectrum activity against preclinical tumor models. Carzelesin demonstrated superior therapeutic activity as compared with adozelesin against both rodent tumors and a limited series of xenografts. In this study we focused on two tumor histiotypes: colon adenocarcinomas,

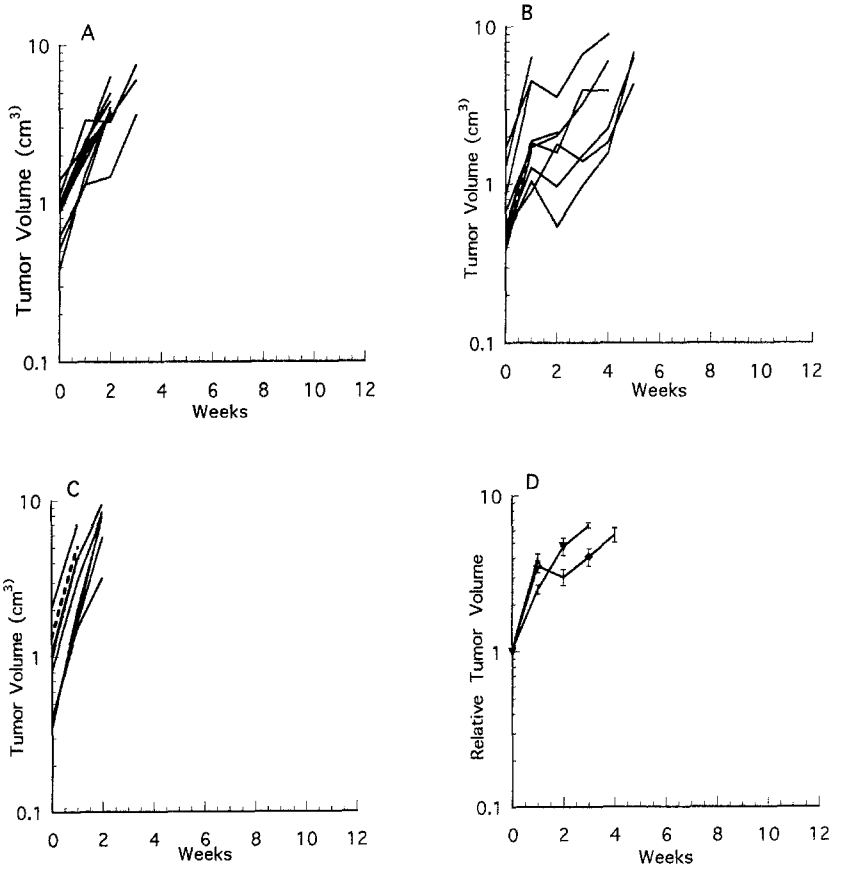


Fig. 4A–D Responses of VRC_5/TOPO colon adenocarcinoma xenografts to carzelesin. Mice received **A** vehicle or carzelesin at **B** 0.5 or **C** 0.25 mg/kg i.v as a single administration. **A–C** Growth patterns of individual tumors. **D** Mean growth curves generated for each treatment group ± SE

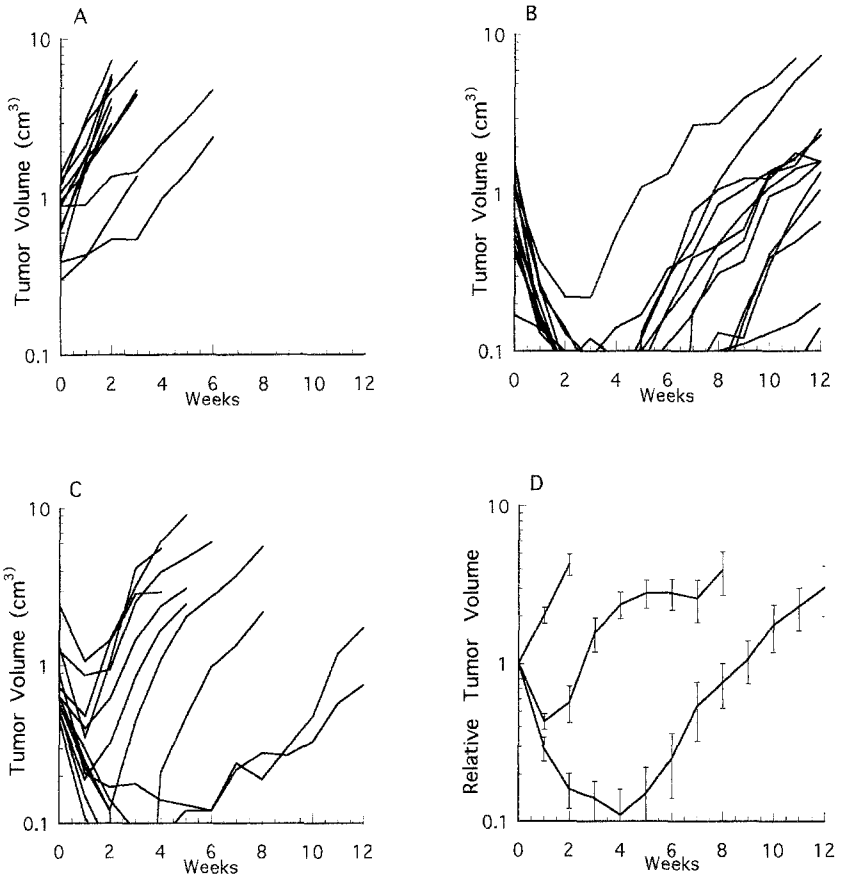


Fig. 5A–D Responses of Rh18/TOPO rhabdomyosarcoma xenografts to carzelesin. Mice received **A** vehicle or carzelesin at **B** 0.5 or **C** 0.25 mg/kg i.v as a single administration. **A–C** Growth patterns of individual tumors. **D** Mean growth curves generated for each treatment group ± SE

representative of chemorefractory epithelial cancers, and childhood rhabdomyosarcomas, typically highly chemoresponsive to alkylating agents. Previous studies using these xenograft models have shown that use of criteria similar to those used to measure objective responses in a clinical situation are most valuable in identifying active new agents [8]. Consequently, to parallel more closely a clinical situation, relatively advanced tumors have been treated and objective responses have been used as meaningful surogate endpoints of drug efficacy.

Previous studies using rodent tumor and xenograft models have indicated that the efficacy of CPI analogues was relatively schedule-independent [13], a single administration being equally active as compared with several split-dose schedules examined. In the present studies, carzelesin was therefore given as a single administration and, consequently, this may not be an optimal schedule for therapeutic utility. Furthermore, it would be anticipated that treatment could be repeated every 3 or 4 weeks in a clinical situation. Carzelesin caused reproducible complete regression of VRC_5 colon carcinomas and partial responses of SJC_3A xenografts. At the highest dose level used, carzelesin significantly inhibited the growth of GC_3 and ELC_2 tumors without causing volume regressions. At 0.25 mg/kg, carzelesin continued to demonstrate marked activity against VRC_5 tumors but no longer exerted a significant effect on SJC_3A or GC_3 xenografts.

Carzelesin demonstrated greater therapeutic activity against xenografts derived from untreated childhood rhabdomyosarcomas, causing complete regressions of Rh30 and IRS56 tumors at the highest dose level. These tumors were highly volume-responsive, regressing rapidly after treatment. This rapid regression rate may have contributed to the relatively high level of toxicity observed in these groups of mice, which accounted for 12 of the 14 deaths occuring among the 201 tumor-bearing mice treated at this dose level. Noteworthy though, is that the effect of carzelesin was markedly reduced at lower dose levels, suggesting a relatively narrow range of effective doses. Resistance to CPI analogues has been shown to be associated with P-glycoprotein expression in multidrug-resistant (MDR) cell lines [16]. However, the pattern of resistance against the panel of xenografts examined would suggest a relatively complex pattern of resistance that does not necessarily cosegregate with the MDR phenotype. For example, two of the colon lines quite responsive to carzelesin (VRC_5, SJC_3A) are completely resistant to drugs such as vincristine and doxorubicin (unpublished results). Conversely, two rhabdomyosarcoma lines selected for primary resistance to vincristine remained as sensitive to carzelesin as their vincristine-sensitive parental lines. The Rh28/LPAM xenograft, selected for

resistance to the bifunctional alkylating agent melphalan, was also as sensitive as the parental Rh28 tumor.

Results obtained with the Rh28 alveolar rhabdomyosarcoma and its melphalan-resistant derivative are of interest for several reasons. First, Rh28 tumors have generally been highly responsive to several classes of chemotherapeutic agents [1, 5–7), particularly bifunctional alkylating agents such as melphalan, cyclophosphamide, and ifosfamide ([7]; unpublished data). Thus, the relative insensitivity of Rh28 tumors to carzelesin was surprising and may indicate a potentially different spectrum of antitumor activity as compared with that of classic alkylating agents. The melphalan-resistant subline Rh28/LPAM demonstrates a complex cross-resistance pattern. Rh28/LPAM has been shown to be cross-resistant to vincristine, etoposide (VP-16), the topoisomerase I inhibitor topotecan and, to a lesser extent, CPT-11 [2,4], as well as the intercalating agent DMP 840 [3]. That carzelesin maintained full, albeit limited, activity against Rh28/LPAM suggests that in situ resistance to CPI drugs may be complex and not necessarily restricted to the P-glycoprotein-mediated phenotype. This conjecture is supported by the cross-resistance pattern observed in two tumor lines selected for resistance to the topoisomerase I inhibitor topotecan. VRC_5 colon adenocarcinoma xenografts were completely resistant to carzelesin, whereas Rh18/TOPO tumors were as sensitive as their parental lines. Further study of these tumor lines selected for resistance to topoisomerase I inhibitors may be of value in elucidating in situ mechanisms associated with carzelesin resistance.

In summary, carzelesin has significant activity against both colon tumors and rhabdomyosarcoma xenografts. However, the activity was observed only at the highest dose levels, and the effective dose range was consequently narrow. Our data would indicate that close inspection of the pharmacokinetics of carzelesin in mice and in phase I clinical trials may assist in determining (a) the likelihood of this agents having significant activity against adult carcinomas and (b) whether this agent should be evaluated against pediatric malignant solid tumors.

References

1. Horton JK, Houghton PJ, Houghton JA (1987) Reciprocal cross-resistance in human rhabdomyosarcomas selected in vivo for primary resistance to vincristine and L-phenylalanine mustard. Cancer Res 47:288
2. Houghton PJ, Cheshire PJ, Hallman JD, Bissery MC, Mathieu-Boué A, Houghton JA (1993) Therapeutic efficacy of the topoisomerase I inhibitor 7-ethyl-10-(4[1-piperidino]-1-piperidino)-carbonyloxycamptothecin against human tumor xenografts: lack of cross-resistance in vivo in tumors with acquired resistance to the topoisomerase I inhibitor 9-dimethylaminomethyl-10-hydroxycamptothecin. Cancer Res 53:2823
3. Houghton PJ, Cheshire PJ, Hallman JD, Gross JL, McRipley RJ, Sun J-H, Behrens CH, Dexter DL, Houghton JA (1994) Evaluation of a novel bis-naphthalimide anticancer agent, DMP840, against human xenografts derived from adult, juvenile, and pediatric cancers. Cancer Chemother Pharmacol 33:265
4. Houghton PJ, Cheshire PJ, Myers L, Stewart CF, Synold TW, Houghton JA (1992) Evaluation of 9-dimethylaminomethyl-10-hydroxycamptothecin against xenografts derived from adult and childhood solid tumors. Cancer Chemother Pharmacol 31:229
5. Houghton JA, Cook RL, Lutz PJ, Houghton PJ (1984) Childhood rhabdomyosarcoma xenografts: response to DNA interacting agents and agents used in current clinical therapy. Eur J Cancer Clin Oncol 20:955
6. Houghton JA, Cook RL, Lutz PJ, Houghton PJ (1985) Melphalan: a potential new agent in the treatment of childhood rhabdomyosarcoma. Cancer Treat Rep 69:9
7. Houghton PJ, Horton JK, Houghton JA (1991) Drug sensitivity and resistance in the xenograft model. In: Maurer HM, Ryman FB, Pochedly C (eds) Rhabdomyosarcoma and related tumors in children and adolescents, CRC Boca Raton, p 187
8. Houghton JA, Houghton PJ (1987) The suitability and use of human tumor xenografts. In: Kallman RF (ed) Rodent tumor models in experimental cancer therapy. Pergamon, New York, p 199
9. Houghton PJ, Houghton JA, Myers L, Cheshire PJ, Howbert JJ, Grindey GB (1989) Evaluation of *N*-(5-indanylsulfonyl)-*N'*-(4-chlorophenyl) urea against xenografts of pediatric rhabdomyosarcoma. Cancer Chemother Pharmacol 25:84
10. Houghton PJ, Shapiro DN, Houghton JA (1991) Rhabdomyosarcoma, from the laboratory to the clinic. Pediatr Clin North Am 38:349
11. Houghton JA, Taylor DM (1978) Growth characteristics of human colorectal tumours during serial passage in immune-deprived mice. Br J Cancer 37:213
12. Kelly RC, Gebhard I, Wicnienski N, Aristoff P, Johnson PD, Martin DG (1987) Coupling of cyclopropapyrroloindole (CPI) derivatives. The preparation of CC-1065, ent-CC-1065, and analogs. J Am Chem Soc 109:6837
13. Li LH, DeKoning TF, Kelly RC, Krueger WC, McGovren JP, Padbury GE, Petzold GL, Wallace TL, Ouding RJ, Prairie MD, Gebhard I (1992) Cytotoxicity and antitumor activity of carzelesin, a prodrug cyclopropylpyrroloindole analogue. Cancer Res 52:4904
14. Li LH, Kelly R, Warpehoski MA, McGovren JP, Gebhard I, DeKoning TF (1991) Adozelesin, a selected lead among cyclopropylpyrroloindole analogs of the DNA-binding antibiotic, CC-1065. Invest New Drugs 9:137
15. Warpehoski MA, Gebhard I, Kelly RC, Krueger WC, Li LH, McGovren JP, Prairie MD, Wicnienski N, WaRenga W (1988) Stereo electronic factors influencing the biological activity and DNA interaction of synthetic antitumor agents modeled on CC-1065. J Med Chem 31:590
16. Zsido TJ, Beerman TA, Meegan RL, Woynarowski JM, Baker RM (1992) Resistance of CHO cells expressing P-glycoprotein to cyclopropylpyrroloindole (CPI) alkylating agents. Biochem Pharmacol 43:1817