Cancer Chemother Pharmacol (2000) 45: 345–349

ORIGINAL ARTICLE

**Henry S. Friedman · Robert C. Castellino
Gertrude B. Elion · Stephen T. Keir · Peter J. Houghton
Stewart P. Johnson · Darell D. Bigner**

Schedule-dependent activity of irinotecan plus BCNU against malignant glioma xenografts

Received: 13 July 1999 / Accepted: 25 October 1999

Abstract *Purpose*: To further evaluate the activity of irinotecan (CPT-11) plus 1,3-bis-(chloroethyl)-1-nitrosourea (BCNU) in the treatment of central nervous system tumor-derived xenografts in athymic nude mice. *Methods*: We report studies evaluating the schedule-dependence of this regimen in the treatment of the malignant glioma xenograft D-54 MG. *Results*: The combination of BCNU and CPT-11 showed the highest enhancement index (2.0–3.3) when BCNU was given on day 1 and CPT-11 was given on days 1–5 and 8–12. Delay of CPT-11 administration to day 3 or day 5 substantially decreased activity with enhancement indices of 1.6–1.8 and 0.6–1.0, respectively. Delay of BCNU administration to day 8 also reduced the CPT-11 activity with enhancement indices of 1.2–1.4. *Conclusions*: These results suggest that the presence of a BCNU-induced adduct or possibly crosslink prior to administration of CPT-11 is critical for enhanced activity. Although the mechanism of this enhancement is not currently known, a phase I trial of CPT-11 plus BCNU for adults with recurrent malignant glioma based on these results is in progress.

H. S. Friedman (✉) · R. C. Castellino · S. T. Keir
S. P. Johnson · D. D. Bigner
Department of Surgery, Duke University Medical Center,
Box 3624, South Hospital, Durham, NC 27710, USA
Tel.: +1-919-684-5301; Fax: +1-919-684-6674

G. B. Elion
Department of Pharmacology,
Duke University Medical Center,
Durham, North Carolina, USA

P. J. Houghton
Department of Molecular Pharmacology,
St. Jude Children's Research Hospital,
Memphis, Tennessee, USA

H. S. Friedman · D. D. Bigner
Department of Pathology,
Duke University Medical Center,
Durham, North Carolina, USA

Key words Nitrosourea · CPT-11 · Glioma

Introduction

Malignant neoplasms of the central nervous system (CNS) are a major cause of death in children and adults [1, 3, 19]. Current treatment options of these malignancies include surgery, radiotherapy, and/or chemotherapy. Chemotherapy has garnered increasing attention with the realization that, at the time of initial diagnosis, most malignant CNS tumors have already metastasized beyond the primary site. The goal of combination chemotherapy is selective destruction of rapidly proliferating tumor cells without damage to normal organs and prevention of emergence of drug-resistant tumor cells.

Irinotecan (CPT-11) is a potent topoisomerase I inhibitor, which stabilizes the covalent bond formed between topoisomerase I and DNA during DNA synthesis, preventing relegation of the DNA and ultimately leading to cell death [8, 13, 18]. CPT-11 has been shown to be active against CNS tumor xenografts in athymic nude mice [12] and recurrent malignant glioma in adults [7]. Alkylating agents such as 1,3-bis-(chloroethyl)-1-nitrosourea (BCNU) produce their antineoplastic effect by first covalently binding one alkyl group to a strand of DNA, with subsequent formation of a DNA interstrand crosslink, which is the lethal lesion [14, 20, 22].

Recent studies with human glioma xenograft D-54 MG have shown that when CPT-11 is given in combination with BCNU, antitumor activity is striking with a greater than additive effect at all doses tested [4]. The mechanism that accounts for this increased activity, however, remains unclear. We now report studies that indicate the importance of schedule to the interaction between CPT-11 and BCNU and suggest mechanism(s) for the observed increase in antineoplastic activity.

Materials and methods

Animals/subcutaneous xenograft transplantation

Male and female athymic BALB/c mice (nu/nu genotype, 6 weeks or older) were used in studies and were maintained as described previously [2]. D-54 MG, an xenograft derived from a human malignant glioma, was used for all studies as previously described [10]. Xenografts were transplanted subcutaneously into the right flanks of animals as described previously [6].

Drugs/tumor measurements

CPT-11 was provided by Pharmacia & Upjohn (Global Distribution Center, Kalamazoo, Mich., USA). BCNU was provided by the Pharmaceutical Research Division of the NCI (Bethesda, Md., USA). Tumors were measured twice a week with a hand-held vernier caliper (Scientific Products, McGraw, Ill., USA). Tumor volume was calculated according to the formula: $[(\text{width})^2 \times (\text{length})]/2$.

Xenograft therapy

In the first set of studies, CPT-11 was given to mice on days 1–5 and 8–12 via intraperitoneal (i.p.) injection at 30 mg/m^2 per dose (10 mg/kg per dose) in 10% dimethylsulfoxide (DMSO) in 0.9% saline, which represents 25% of the dose lethal to 10% of treated animals (LD_{10}). BCNU was given to mice either on day 1 or on day 8 via i.p. injection at 50 mg/m^2 (16.67 mg/kg) in 10% ethanol in 0.9% saline, which represents 50% of the LD_{10}. On day 1 of the study, CPT-11 was administered 5 h after BCNU administration. Groups of ten randomly selected mice began drug treatment when the median tumor volume exceeded 200 mm^3 and were compared with control animals receiving the drug vehicle.

In the second set of studies, BCNU was given to mice on day 1 via i.p. injection at 50 mg/m^2. CPT-11 was given to mice using one of three schedules: (1) days 1–5 and 8–12, (2) days 3–7 and 10–14, or (3) days 5–9 and 12–16 via i.p. injection at 10 mg/kg per dose. CPT-11 was administered 5 h after BCNU administration. Groups of ten randomly selected mice began drug treatment when median tumor volume exceeded 200 mm^3 and were compared with control animals receiving the drug vehicle.

Assessment of response

Response of subcutaneous xenografts was assessed by delay in tumor growth and by tumor regressions. Growth delay (T-C) is the difference in days between median time required for treated (T) and control (C) animals to reach a volume five times greater than that measured at the start of treatment. Tumor regression is defined as a decrease in tumor volume over two successive measurements. Statistical analyses were performed using a Wilcoxon rank order test for growth delay and the Fisher's exact test for tumor regression as described previously [6].

Results

The initial set of studies was conducted with CPT-11 administered on days 1–5 and 8–12 and BCNU administered on either day 1 or day 8. The combination of CPT-11 and BCNU on these schedules produced a substantial increase in antitumor activity when BCNU was administered on day 1 but much less when BCNU was administered on day 8 (Table 1). The second set of studies was conducted with BCNU administration on day 1 and CPT-11 administration starting on day 1, 3, or 5. The activity of CPT-11 plus BCNU in combination was dramatically decreased as the CPT-11 was delayed, with only additive values or less when CPT-11 was administered on day 5 (Table 1).

The enhancement index, defined as the growth delay produced by combination therapy of BCNU plus CPT-11 divided by the sum of the growth delays of BCNU plus CPT-11, was highest for BCNU administered on day 1 and CPT-11 administered on days 1–5 and 8–12, with values of 2.0–3.3. Delay of the start of CPT-11 to day 3 or day 5 decreased the enhancement index to 1.6–1.8 and 0.6–1.0, respectively. Delay of BCNU administration to day 8 also reduced the enhancement index to 1.2–1.4.

Discussion

The role of chemotherapy in the treatment of high-grade glioma has remained controversial. Although several small studies suggested a benefit from chemotherapy [9, 21], Brain Tumor Study Group (BTSG) 7201 was the first large study to demonstrate a possible additive effect of BCNU with radiation [23]. The addition of post-radiation BCNU (80 mg/m^2 × 3 days every 8 weeks) produced a median survival of 51 weeks compared with 36 weeks for radiotherapy alone. The difference in survival was not statistically significant, but provocative enough to provide the foundation of BTSG 7501. This study treated all patients with whole brain radiotherapy and then randomized them to treatment on one of four arms including high-dose methylprednisone with or without BCNU or procarbazine [11]. Patients treated on the BCNU arm had a statistically significant increase in median survival over treatment on the high-dose methylprednisone arm (50 vs 40 weeks). Although the BTSG concluded that BCNU in the post-radiation setting benefited patients with high-grade glioma, it is important to realize that it is possible that the patients on no chemotherapy, high-dose methylprednisone "control" suffered a high number of steroid-induced complications, reducing median survival, compared with the BCNU arm [5].

Recent data has been generated from Northern California Oncology Group (NCOG) trial 6G61, which randomized patients with high-grade glioma to whole brain radiotherapy with hydroxyurea plus either BCNU or PCV (procarbazine, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), vincristine) [15]. The analysis of all patients revealed that there was a non-statistically significant increase in survival favoring PCV [16]. However, a subgroup analysis by Levin et al. [17] revealed that patients with anaplastic astrocytoma survived nearly twice as long when treated with PCV compared with those treated with BCNU (151.1 vs 82.1 weeks, respectively). Based on this report, PCV has become the standard post-radiation therapy for patients with anaplastic astrocytoma.

Table 1 Treatment of nude mice bearing D-54 MG xenografts growing subcutaneously with BCNU, CPT-11 or BCNU plus CPT-11. *BCNU* 1,3-bis-(chloroethyl)-1-nitrosourea, *CPT*-11 irinotecan

Experiment	Day of BCNU[a]	Administration CPT-11[b]	T-C[c]	Enhancement index[d]	Regressions[e]	Toxic deaths
1	1		2.0		0	0
		1–5, 8–12	4.5		1	4
	1	1–5, 8–12	15.0*	2.0	5	1
2	1		0.9		0	0
		1–5, 8–12	3.3		2	0
	1	1–5, 8–12	14.0*	3.3	4	0
3	1		2.1		0	0
		1–5, 8–12	5.6*		0	0
	1	1–5, 8–12	16.6*	2.2	7	0
4	1		1.3		0	0
		1–5, 8–12	9.6*		0	0
	1	1–5, 8–12	21.4*	2.0	8	0
5	8		2.1		0	0
		1–5, 8–12	16.2*		7	0
	8	1–5, 8–12	26.2*	1.4	10	0
6	8		1.4		0	0
		1–5, 8–12	9.6*		0	0
	8	1–5, 8–12	13.1*	1.2	3	0
7	1		0.7		0	0
		3–7, 10–14	6.6*		2	2
	1	3–7, 10–14	12.7*	1.7	5	2
8	1		0.9		0	0
		3–7, 10–14	4.4*		0	0
		3–7, 10–14	9.6*	1.8	3	0
9	1	3–7, 10–14	2.1		0	0
			2.7		0	0
	1	3–7, 10–14	7.7*	1.6	1	0
10	1		2.1		0	0
		5–9, 12–16	2.6		0	0
	1	5–9, 12–16	4.6*	1.0	0	0
11	1		1.3		0	0
		5–9, 12–16	9.2*		1	0
	1	5–9, 12–16	5.8*	0.6	0	0

[a] BCNU was administered intraperitoneally on the day indicated at a dose of 50 mg/m^2 in 10% ethanol

[b] CPT-11 was administered i.p. on the days indicated at a dose of 30 mg/m^2 in 10% dimethylsulfoxide

[c] *T-C*, the difference in days between the median time for treated (*T*) and control (*C*) animals to reach a volume five times greater than that measured at the start of treatment

[d] Enhancement index is defined as the growth delay (*T-C*) produced by combination therapy with BCNU plus CPT-11 divided by the sum of the growth delays of BCNU plus CPT-11 (T-C of BCNU plus T-C of CPT-11)

[e] Tumor regression is defined as a decrease in tumor volume over two successive measurements

* *P* value versus controls < 0.01

The role of CPT-11 in the treatment of malignant glioma is unclear. Initial laboratory studies using CPT-11 against human CNS tumor-derived xenografts in athymic nude mice demonstrated prodigious antitumor activity [12] and led directly to a phase II trial of this agent in adults with recurrent malignant glioma. The drug was active, albeit with only eight partial responses in 48 patients with glioblastoma multiforme [7]. However, these patients were obviously under-dosed, since the concomitant use of the anticonvulsants Dilantin or Tegretol dramatically enhanced clearance of the active metabolite SN-38. These patients were treated with CPT-11 at a dose of 125 mg/m^2, whereas recent studies indicate that for patients receiving these anticonvulsants, the maximal tolerated dose is still undefined but higher than 267 mg/m^2 (S. Grossman, personal communication).

The initial studies combining CPT-11 plus alkylating agents revealed marked enhancement of antitumor activity when BCNU plus CPT-11 were both administered [4]. However, neither the optimal regimen nor the mechanism of this enhancement was evaluated. The current studies strongly suggest that maximal enhancement of antitumor activity without an increase in toxicity is seen when BCNU and CPT-11 are started on the same day. A 2-day delay of the CPT-11 produces a drop in activity, whereas a 4-day delay may actually decrease activity below additive values. Similarly, delay of BCNU to day 8 produces a marked drop in activity, despite CPT-11 being continued for days 8–12. The explanation for these results is unclear. Initially we favored the hypothesis that CPT-11-induced inhibition of topoisomerase I precluded repair of BCNU-induced DNA interstrand crosslinks. Delay of the use of CPT-11 resulting in less enhancement of activity could then have been due to cytotoxicity produced by the crosslinks whose formation peak at 12–24 h. However, delay of the BCNU to day 8 also reduces the activity. Furthermore,

use of noncrosslinking alkylators, particularly temozolomide in combination with CPT-11 also resulted in enhanced antitumor activity (data not shown).

An alternative hypothesis which remains unproved is that an adduct on the O^6 position of guanine enhances CPT-11 activity. This could be explained by a BCNU-induced increase in topoisomerase I activity or recruitment of topoisomerase I to its binding site on DNA. Delay of the BCNU to day 8 might be less effective if prior treatment with CPT-11 reduces the BCNU effect on topoisomerase. Delay of the CPT-11 might be less effective since the BCNU-induced adduct could be repaired or converted by a crosslink by the time CPT-11 is administered. This hypothesis remains speculative but is currently being evaluated in additional laboratory studies.

Despite a lack of mechanistic explanation for the observed results, the enhancement of antiglioma activity seen when BCNU and CPT-11 are both initiated on day 1 is dramatic and reproducible. This observation should be evaluated in other histologies, since there is no reason to believe it is histology-specific. However, it is possible that differences between murine and humans, including the duration of CPT-11 exposure, rate of metabolic conversion, and relative doses administered, may result in a less favorable interaction of CPT-11 and BCNU in humans. Nevertheless, on the basis of the preclinical studies to date, we have initiated a phase I trial of CPT-11 plus BCNU in which both agents are started on day 1 as therapy for adults with recurrent malignant glioma.

Acknowledgement Karin Jane Stern provided editorial assistance.

References

1. Bleyer WA (1992) The impact in the United States and the world of center nervous system cancer during childhood. In: Packer RJ, Bleyer WA, Pochedly C (eds) Pediatric neuro-oncology. Harwood Academic Publishers, Philadelphia, p 1
2. Bullard DE, Bigner DD (1979) Heterotransplantation of human craniopharyngiomas in athymic "nude" mice. Neurosurgery 4: 308
3. Chang CH, Horton J, Schoenfeld D, Salazer O, Perez-Tamayo R, Kramer S, Weinstein A, Nelson JS, Tsukada Y (1983) Comparison of postoperative radiotherapy and combined postoperative radiotherapy and chemotherapy in the multidisciplinary management of malignant gliomas. A joint Radiation Therapy Oncology Group and Eastern Cooperative Oncology Group study. Cancer 52: 997
4. Coggins CA, Elion GB, Houghton PJ, Hare CB, Keir S, Colvin OM, Bigner DD, Friedman HS (1998) Enhancement of irinotecan (CPT-11) activity against central nervous system tumor xenografts by alkylating agents. Cancer Chemother Pharmacol 41: 485
5. Fine HA (1994) The basis for current treatment recommendations for malignant gliomas. J Neurooncol 20: 111
6. Friedman HS, Colvin OM, Skapek SX, Ludeman SM, Elion GB, Schold SC Jr, Jacobsen PF, Muhlbaier LH, Bigner DD (1988) Experimental chemotherapy of human medulloblastoma cell lines and transplantable xenografts with bifunctional alkylating agents. Cancer Res 48: 4189
7. Friedman HS, Petros WP, Friedman AH, Schaaf LJ, Kerby T, Lawyer J, Parry M, Houghton PJ, Lovell S, Rasheed K, Cloughesy T, Stewart ES, Colvin OM, Provenzale JM, McLendon RE, Bigner DD, Cokgor I, Haglund M, Rich J, Ashley DM, Malczyn J, Elfring GL, Miller LL (1999) Irinotecan therapy in adults with recurrent or progressive malignant glioma. J Clin Oncol 17: 1516
8. Fukuoka M, Niitani H, Suzuki A, Motomiya M, Hasegawa K, Nishiwaki Y, Kuriyama T, Ariyoshi Y, Negoro S, Masuda N, Nakajima S, Taguchi T (1992) A phase II study of CPT-11, a new derivative of camptothecin, for previously untreated non-small cell lung cancer. J Clin Oncol 10: 16
9. Garrett MJ, Hughes HJ, Freeman LS (1978) A comparison of radiotherapy alone with radiotherapy and CCNU in cerebral glioma. Clin Oncol 4: 71
10. Giard DJ, Aronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP (1973) In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J Natl Cancer Inst 51: 1417
11. Green SB, Byar DP, Walker MD, Pistenman DA, Alexander E Jr, Batzdorf U, Brooks WH, Hunt WE, Mealey J Jr, Odom GL, Paoletti P, Ransohoff J II, Robertson JT, Selker RG, Shapiro WR, Smith KR Jr, Wilson CB, Strike TA (1983) Comparisons of carmustine, procarbazine and high-dose methylprednisolone as additions to surgery and radiotherapy for the treatment of malignant glioma. Cancer Treat Rep 67: 121
12. Hare CB, Elion GB, Houghton PJ, Houghton JA, Keir S, Marcelli SL, Bigner DD, Friedman HS (1997) Therapeutic efficacy of topoisomerase I inhibitor 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin against pediatric and adult central nervous system tumor xenografts. Cancer Chemother Pharmacol 39: 187
13. Houghton PJ, Cheshire PJ, Hallman JC, Bissery MC, Mathieu-Boue A, Houghton JA (1993) Therapeutic efficacy of topoisomerase I inhibitor 7-ethyl-10-(4-[1-piperidino]-1-piperidino-carbonyloxy-camptothecin against human tumor xenografts: lack of cross resistance in vivo in tumors with acquired resistance to topoisomerase I inhibitor 9-dimethylaminomethyl-10-hydroxycamptothecin. Cancer Res 53: 2823
14. Houghton PJ, Cheshire PJ, Hallman JD II, Lutz L, Friedman HS, Danks MK, Houghton JA (1995) Efficacy of topoisomerase I inhibitors, topotecan and irinotecan, administered at low dose levels in protracted schedules to mice bearing xenografts of human tumors. Cancer Chemother Pharmacol 36: 393
15. Levin VA, Wara WM, Davis RL, Vestnys P, Resser KJ, Yatsko K, Nutik S, Gutin PH, Wilson CB (1985) Phase III comparison of BCNU and the combination of procarbazine, CCNU, and vincristine administered after radiotherapy with hydroxyurea for malignant gliomas. J Neurosurg 63: 218
16. Levin VA, Wara WM, Davis RL, Silver P, Resser KJ, Yatsko K, Nutik S, Gutin PH, Wilson CB (1986) Northern California Oncology Group protocol 6G91: response to treatment with radiation therapy and seven-drug chemotherapy in patients with glioblastoma multiforme. Cancer Treat Rep 70: 739
17. Levin VA, Silver P, Hannigan J, Wara WM, Gutin PH, Davis RL, Wilson CB (1990) Superiority of post-radiotherapy adjuvant chemotherapy with CCNU, procarbazine, and vincristine (PCV) over BCNU for anaplastic gliomas: NCOG 6G61 final report. Int J Radiat Oncol Biol Phys 18: 321
18. Ohno R, Okada K, Masaoka T, Kuramoto A, Arima T, Yoshida Y, Ariyoshi H, Ichimaru M, Sakai Y, Oguro M, Ito Y, Morishima Y, Yokomaku S, Ota K (1990) An early phase II study of CPT-11: a new derivative of camptothecin, for the treatment of leukemia and lymphoma. J Clin Oncol 8: 1907
19. Shapiro WR (1986) Therapy of adult malignant brain tumors. What have the clinical trials taught us? Semin Oncol 13: 38
20. Slichenmyer WJ, Rowinsky EK, Grochow LB, Kaufmann SH, Donehower RC (1994) Camptothecin analogues: studies from The Johns Hopkins Oncology Center. Cancer Chemother Pharmacol 34: 53
21. Solero CL, Monfardini S, Brambilla C, Vaghi A, Valagussa P, Morello G, Bonadonna G (1979) Controlled study with BCNU vs. CCNU as adjuvant chemotherapy following surgery plus

radiotherapy for glioblastoma multiforme. Cancer Clin Trials 2: 43

22. Von Hoff DD, Burris HA III, Eckardt J, Rothenberg M, Fields SM, Chen SF, Kuhn JG (1994) Preclinical and phase I trials of topoisomerase I inhibitors. Cancer Chemother Pharmacol 34: 41
23. Walker MD, Green SB, Byar DP, Alexander E Jr, Batzdorf U, Brooks WH, Hunt WE, MacCarty CS, Mahaley MS Jr, Mealey J Jr, Owens G, Ransohoff J II, Robertson JT, Shapiro WR, Smith KR Jr, Wilson CB, Strike TA (1980) Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. N Engl J Med 303: 1323