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Therapeutic efficacy of the topoisomerase I inhibitor 7-ethyl-10-(4-[1-piperidino]-1-piperidino)-carbonyloxy-camptothecin against pediatric and adult central nervous system tumor xenografts

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Abstract Therapy of patients with malignant central nervous system tumors is frequently unsuccessful, reflecting limitations of current surgical, radiotherapeutic, and pharmacotherapeutic treatments. The camptothecin derivative irinotecan (CPT-11) has been shown to possess antitumor activity in phase II trials for patients with carcinoma of the lung, cervix, ovary, colon, or rectum and for patients with non-Hodgkin's lymphoma. The current study was designed to test the efficacy of the drug against a panel of human tumor xenografts derived from adult and pediatric central nervous system malignancies. Tumors included childhood high-grade gliomas (D-212 MG, D-456 MG), adult high-grade gliomas (D-54 MG, D-245 MG), medulloblastomas (D341 Med, D487 Med), ependymomas (D528 EP, D612 EP), and a rhabdomyosarcoma (TE-671), as well as sublines with demonstrated resistance to busulfan (D-456 MG (BR)), cyclophosphamide (TE-671 CR), procarbazine (D-245 MG (PR)) or melphalan (TE-671 MR), growing subcutaneously and intracranially in athymic nude mice. In replicate experiments, CPT-11 was given at a dosage of 40 mg/kg per dose via intraperitoneal injection in 10% dimethylsulfoxide on days 1–5 and 8–12, which is the dosage lethal to 10% of treated animals. CPT-11 produced statistically significant ($P < 0.001$) growth delays in all subcutaneous xenografts tested, including those resistant to busulfan, cyclophosphamide, procarbazine, and melphalan, with growth delays ranging from 21.3 days in D487 Med to 90 + days in several tumor lines. Further, tumor regression was evident in every treated animal bearing a subcutaneous tumor, with some xenografts yielding complete tumor regression. Statistically significant ($P < 0.001$) increases in survival were demonstrated in the two intracranial xenografts – D341 EP (73.0% increase) and D-456 MG (114.2% increase) – treated with CPT-11. These studies demonstrate that, of over 40 drugs evaluated in this laboratory, CPT-11 is the most active against central nervous system xenografts and should be advanced to clinical trial as soon as possible.

Key words CPT-11 · Topoisomerase I · CNS tumors · Xenograft · Camptothecin

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Introduction

The most common solid neoplasms in children under the age of 15 years are tumors of the central nervous system (CNS) [2], including medulloblastoma, astrocytoma, and glioblastoma multiforme. The treatment of these tumors remains a frustrating therapeutic challenge [8], with only limited success being achieved with conventional surgical, radiotherapeutic, and chemotherapeutic interventions. A substantial number of patients with small, nonmetastatic, completely resected medulloblastoma may be cured with radiotherapy; however, the majority of children with medulloblastoma will ultimately die of progressive disease [9]. Glioblastoma multiforme presents an even greater therapeutic challenge, as fewer than 30% of children

survive more than 3 years from diagnosis [4]. Although chemotherapy offers some hope of increasing these dismal prognoses, there are problems peculiar to pharmacotherapy that must be addressed, including limitations on the activity of the drug against a particular tumor histology, delivery of the drug to privileged intracranial (i.c.) sites, *de novo* presence or emergence of drug resistance, and sensitivity of the normal brain tissue to irreversible damage from any therapeutic modality. The limited number of chemotherapeutic agents available that possess substantial activity against brain tumors supports the identification of novel agents for treatment as well as the development of therapeutic strategies for overcoming or bypassing drug resistance.

With the goal of finding new antineoplastic agents, the National Cancer Institute undertook an extensive program of screening natural products during the 1950s. One agent identified as having activity against L1210 murine leukemia was an extract from the Chinese tree *Camptotheca acuminata;* it was called camptothecin (CPT) [15]. Subsequent studies with this compound showed it to work by interacting with the nuclear enzyme topoisomerase I (topo I). Topo I normally functions in the nucleus to relax torsional strain on double-stranded DNA – a strain created during the unwinding of the helical molecule during replication. To accomplish this, topo I introduces a single-strand break in the phosphodiester backbone of the molecule, allows the intact strand to pass through the break, and then re-anneals the broken strand [15]. CPT stabilizes the adducts containing topo I, which is covalently bound to the DNA during the catalytic cycle of the enzyme and prevents the relegation of the broken single strand of DNA. These single-strand breaks are irreversibly converted into double-strand breaks through the action of advancing replication forks – a cytotoxic event for the cell [15].

CPT-11 (irinotecan) is a water-soluble analog of camptothecin that demonstrates significant antitumor activity against a broad spectrum of tumor xenografts in vivo when given by intraperitoneal (i.p.), intravenous (i.v.), or oral routes [12, 15]. It is a prodrug converted to the active metabolite SN-38 [15]. Phase I and phase II clinical studies done primarily in Japan and France have demonstrated activity of CPT-11 against ovarian, colon, breast, gastric, and nonsmall-cell and small-cell lung neoplasms, with a dose-limiting toxicity of neutropenia and gastrointestinal disturbances [15].

Building on this knowledge, we tested CPT-11 against a panel of human tumor xenografts derived from adult and pediatric CNS malignancies and transplanted in athymic mice. Tumors tested included childhood high-grade gliomas (D-212 MG, D-456 MG), adult high-grade gliomas (D-54 MG, D-245 MG), medulloblastomas (D341 Med, D487 Med), ependymomas (D528 EP, D612 EP), and a rhabdomyosarcoma (TE-671), as well as sublines of these xenografts with laboratory-generated resistance to either busulfan (D-456 MG (BR)), cyclophosphamide (TE-671 CR), melphalan (TE-671 MR), or procarbazine (D-245 MG (PR)). CPT-11 produced statistically significant ($P < 0.001$) growth delays in all subcutaneous (s.c.) xenografts tested, including those resistant to conventional therapy. Further, tumor regression was evident in every animal treated, with some xenografts yielding complete tumor regression. Statistically significant increases ($P < 0.001$) in survival were also demonstrated in the two i.c. xenografts treated with CPT-11.

Materials and methods

Animals

Male and female athymic BALB/c mice (*nu/nu* genotype, 6 weeks or older) were used for all studies and were maintained as described previously [3].

Xenografts

A panel of 13 human CNS tumor-derived xenografts was used for all in vivo studies. The xenografts, described above, were maintained as previously described [11].

Drugs

CPT-11 was provided by Pharmacia & Upjohn (Global Distribution Center, Kalamazoo, Mich.).

Subcutaneous xenograft transplantation

Subcutaneous tumor transplantation into the right flank of the animals was performed as described previously, with inoculation volumes of 50 μl [7].

Intracranial xenograft transplantation

Intracranial tumor transplantation into the right cerebrum was performed as described previously, with inoculation volumes of 10 μl [7].

Tumor measurements

Tumors were measured twice weekly with hand-held vernier calipers (Scientific Products, McGraw, Ill.). Tumor volume was calculated according to the following formula: $[(\text{width})^2 \times (\text{length})]/2$.

Xenograft therapy

In replicate experiments, CPT-11 was given to mice via i.p. injection in a volume of 40 mg/kg per dose in 10% dimethylsulfoxide in saline on days 1–5 and 8–12, which represents the dosage lethal to 10% of treated animals. For s.c. studies, groups of ten randomly selected mice began receiving treatment when the tumor volume was within

the range of 100–500 mm^3 and were compared with control animals receiving no treatment. For i.c. studies, groups of ten randomly selected animals began receiving treatment on the day that represented 50% of the time elapsing between the initial tumor inoculation and the median day of death for mice bearing i.c. tumor and receiving no therapy. Controls received no treatment.

Assessment of response

The response of s.c. xenografts was assessed by delay in tumor growth and by tumor regression. Growth delay, expressed as T − C, was defined as the difference in days between the median time required for tumors in treated (T) and control (C) animals to reach a volume five times greater than that measured at the start of treatment. Tumor regression was defined as a decrease in tumor volume over two successive measurements. Statistical analyses were performed using a personalized SAS statistical analysis program, the Wilcoxon rank order test for growth delay, and Fisher's exact test for tumor regression as described previously [7]. The response of i.c. xenografts was assessed by the percentage increase in median survival. Statistical analysis was performed using the Wilcoxon rank order test as described previously [7].

Results

Toxicity

Among 260 treated animals, 36 deaths were attributable to drug toxicity. The median nadir weight loss was 6.5% among surviving animals. No neurologic toxicity, including seizure activity, was noted.

Subcutaneous xenograft therapy

CPT-11 was active against all tumor lines tested, including those resistant to busulfan, cyclophosphamide, procarbazine, and melphalan (Table 1). Growth delays ranged from 21.3 days in D487 Med to 90 + days in several tumor lines; all values were statistically significant ($P < 0.001$). Tumor regressions were seen in all animals bearing s.c. tumors, with some xenografts yielding complete regression.

Intracranial xenograft therapy

CPT-11 produced statistically significant ($P < 0.001$) increases of 73.0–114.2% in the median survival of mice bearing i.c. tumor xenografts (Table 2). All mice displayed gross evidence of i.c. tumor at the time of death.

Table 1 Effect of CPT-11 treatment on growth of s.c. human CNS xenografts in mice. In replicate experiments, CPT-11 was administered via i.p. injection at a dose of 40 mg/kg in 10% dimethylsulfoxide on days 1–5 and 8–12 to ten mice in each xenograft group

Xenografts	Histology	T − C[a] (days)	Regressions[b]
D-212 MG	Childhood high-grade glioma	81.1	8/8 (2)
		120 +	7/7 (4)
D-456 MG	Childhood high-grade glioma	90 +	9/9 (7)
		90 +	8/8 (5)
D-456 MG (BR)	Childhood high-grade glioma – busulfan resistant	90 +	7/7 (7)
		90 +	9/9 (8)
D-54 MG	Adult high-grade glioma	90 +	8/8 (4)
		90 +	8/8 (6)
D-245 MG	Adult high-grade glioma	25.9	10/10
		27.9	7/7
D-245 MG (PR)	Adult high-grade glioma – procarbazine resistant	33.4	9/9
		33.7	9/9
D341 Med	Medulloblastoma	90 +	7/7 (7)
		90 +	10/10 (10)
D487 Med	Medulloblastoma	21.7	8/8
		21.3	9/9
D528 EP	Ependymoma	101.7	9/9
		105.3	9/9
D612 EP	Ependymoma	59.3	9/9 (1)
		52.2	8/8
TE-671	Rhabdomyosarcoma	35.3	8/8
		40.0	8/8 (1)
TE-671 CR	Rhabdomyosarcoma–cyclophosphamide resistant	32.9	8/8
		32.5	10/10
TE-671 MR	Rhabdomyosarcoma–melphalan resistant	30.9	9/9
		28.2	9/9 (1)

[a] T − C, growth delay in days, was defined as the difference between the median time required for tumors in treated (T) and control (C) animals to reach five times the volume measured at the initiation of treatment. All values, from replicate experiments, are statistically significant ($P < 0.001$) compared with controls

[b] Regression was defined as a decrease in tumor volume over two successive measurements. Numbers in parentheses are the number of complete regressions. All values, from replicate experiments, are statistically significant ($P < 0.001$) compared with controls

Discussion

Camptothecins, including the derivative CPT-11, are an exciting new class of antineoplastic agents with a novel mechanism of action. Early studies have demonstrated the activity of CPT-11 against a wide array of tumor histologies, but never before has the drug been studied against an extensive spectrum of tumors of the CNS. The results presented here show outstanding activity for CPT-11 against every histological type of CNS tumor tested, including medulloblastomas, adult and childhood high-grade gliomas, and ependymomas, as well as against a rhabdomyosarcoma. CPT-11 demonstrated the least activity against D-487 Med, a xenograft derived from a highly clinically resistant medulloblastoma. Nevertheless, in testing this tumor line against a panel of other chemotherapeutic agents

Table 2 Effect of CPT-11 treatment on survival of mice bearing i.c. human CNS xenografts. CPT-11 was give via i.p. injection at a dosage of 40 mg/kg in 10% dimethylsulfoxide on days 1–5 and 8–12 to ten mice in each xenograft group. Results for D341 Med are for replicate experiments

Xenograft	Histology	Median day of death		
		Control	Treated	Increase in median survival (%)[a]
D341 Med	Medulloblastoma	18.5	32	73.0
		21	35.5	69.0
D-456 MG	Childhood high-grade glioma	21	45	114.2

[a] Calculated as the median day of death of ten drug-treated mice minus the median day ten death of ten nontreated mice divided by the median day of death of nontreated mice. All values are statistically significant ($P < 0.001$) compared with controls

including cyclophosphamide, temozolamide, melphalan, busulfan, procarbazine, 1,3-bis(2-chloroethyl) nitrosourea, and another CPT derivative 9-amino CPT, the greatest activity was shown with CPT-11 (data not shown).

The activity of CPT-11 against this broad spectrum of CNS xenografts exceeds the results seen with over 40 other chemotherapeutic agents including alkylators, methylators, antimetabolites, vinca alkyloids, anthracyclines, and L-asparaginase [5, 6]. CPT-11 produced tumor regressions in every mouse bearing an s.c. xenograft, results never before seen with any other antineoplastic drug tested in our program, including other topoisomerase I inhibitors such as topotecan or 9-aminocamptothecin [1].

Of particular interest is the activity of CPT-11 against other CNS xenografts with laboratory-generated resistance to alkylating agents, specifically cyclophosphamide, melphalan, busulfan, and procarbazine. TE-671 MR is a melphalan-resistant subline of the human rhabdomyosarcoma TE-671. Melphalan produces growth delays of 34.0 days against TE-671, but growth delays of only ~ 8 days in TE-671 MR [13, 14]. Mechanisms of resistance to melphalan operational in TE-671 MR include reduced intratumor transport and elevated levels of glutathione [13, 14]. Elevated levels of DNA polymerases α and β, as well as topoisomerase II, are also seen. Cross-resistance to all other alkylators tested has been shown in TE-671 MR. CPT-11 produced growth delays of 35.3 and 40.0 days against TE-671 and 30.9 and 28.2 days against TE-671 MR. These slightly reduced growth delays may reflect the reduced topoisomerase I activity.

TE-671 CR is a cyclophosphamide-resistant subline of TE-671. Cyclophosphamide induces growth delays of 28.2 days against TE-671 and only 13.0 days against TE-671 CR. No obvious mechanism of resistance to cyclophosphamide in TE-671 CR has been identified to date, including alterations is glutathione, glutathione *S*-transferase, or aldehyde dehydrogenase (unpublished data). CPT-11 produced growth delays of 35.3 and 40.0 days against TE-671 and 32.9 and 32.5 days against TE-671 CR.

D-456 MG (BR) is a busulfan-resistant subline of the childhood high-grade glioma D-456 MG [10]. Busulfan produces growth delays of 13.3 to 20 days against the parent line and 5.1 days against the busulfan-resistant xenograft. Although no precise mechanism of resistance to busulfan has been identified, it is interesting that D-456 MG (BR) and D-456 MG (BR) were similarly sensitive to CPT-11, with growth delays of 90 + days being seen in both xenografts.

D-245 MG (PR) is the procarbazine-resistant subline of the adult high-grade glioma xenograft D-245 MG. D-245 MG (PR) is absolutely resistant to all bifunctional or monofunctional alkylators tested despite the absence of O^6-alkylguanine-DNA alkyltransferase activity. We are currently exploring potential mechanisms of resistance to methylators in D-245 MG (PR). Procarbazine produces growth delays of 74.7 days against D-245 MG, but only 2.1 days against D-245 MG (PR). CPT-11, in contrast, produces growth delays of 25.9 and 27.9 days against D-245 MG and 33.4 and 33.7 days against D-245 MG (PR).

Based on these findings, we are designing and implementing a phase II clinical trial for CPT-11 in adult patients with newly diagnosed high-grade gliomas. Current efforts in the laboratory are focused on reducing the dosage of CPT-11 to test the dose-response of the drug. Studies designed to test the effects of combination therapy with CPT-11 and cyclophosphamide are also underway. The demonstrated activity of CPT-11 against tumors of varying histologies, as well as against tumors resistant to other chemotherapeutic agents, suggests a wide spectrum of potential uses for CPT-11 in the clinic.

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