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Activity of 9-dimethylaminomethyl-10-hydroxycamptothecin against pediatric and adult central nervous system tumor xenografts

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Abstract. The activity of dimethylaminomethyl-10-hydroxycamptothecin (topotecan) was evaluated against a panel of xenografts derived from ependymomas (D528 EP, D612 EP), childhood high-grade gliomas (D-456 MG, D-212 MG), adult high-grade gliomas (D-245 MG, D-54 MG), and medulloblastomas (D425 Med) growing s.c. and i.c. (intracranially) in athymic nude mice. Topotecan was given at a dose of 1.9 mg/kg by i.p. injection in 0.9% saline using a volume of 90 ml/m^2 on days 1–5 and 8–12, which represents the dose lethal to 10% of treated animals. Topotecan was active in the therapy of all s.c. xenografts tested, with growth delays ranging from 6.3 days in D-54 MG to 55.7 days in D528 EP. Topotecan produced statistically significant tumor regressions in D425 Med, D-456 MG, D-245 MG, D528 EP, and D612 EP. No tumor regression was seen in any control animal. Statistically significant increases in median survival were seen in the two i.c. xenografts – D-456 MG (28.6% increase) and D-54 MG (39% increase) – treated with topotecan. These studies suggest that topotecan may be an important new addition to the therapy of central nervous system tumors.

Introduction

Therapy of patients with malignant central nervous system (CNS) tumors is frequently unsuccessful, reflecting current limitations in the activity of available antineoplastic agents, the delivery of these agents to at least partially privileged intracranial (i.c.) sites, the emergence or de novo presence of resistance to these agents, and the sensitivity of the brain to irreversible damage from any therapeutic modality [4, 7]. Nevertheless, progress, albeit slow, has been made, particularly in the treatment of neuronal tumors such as medulloblastoma [12]. Alkylating agents such as cyclophosphamide, cisplatin, and melphalan demonstrate marked activity against neuronal tumors [1, 7, 11, 12] as judged by classic radiographic response criteria, but acquired or de novo drug resistance frequently develops and is the harbinger of tumor progression and death. Identification of agents active against glial malignancies is more challenging, with no drug tested to date reliably producing responses in a majority of treated patients [6]. Although mechanisms of drug resistance may be identified and drug sensitivity restored through inhibition of these mechanisms, enhanced antineoplastic activity without enhanced toxicity to normal organs may not be readily achievable due to similar biochemical pathways in tumors and normal organs. Efforts need to be devoted to identification of antineoplastic agents with novel mechanisms of action that are potentially not cross-resistant with clinically available conventional drugs.

Generation of panels of permanent cell lines and xenografts derived from specific malignancies provides the opportunity for histiotype-specific laboratory analysis of the phenotypic and genotypic profile of malignancies and, potentially, an increase in the successful search for active antineoplastic drugs [15, 17, 20]. Previous studies have provided new approaches for therapy of medulloblastoma and adult anaplastic glioma by identifying the activity of vincristine plus cyclophosphamide or melphalan in the treatment of medulloblastoma [9–11] and the activity of azyridinylbenzoquinone (AZQ) in the treatment of high-grade glioma [19]. Unfortunately, models allowing laboratory analysis of other childhood brain tumors, such as ependymoma or high-grade glioma, have not been available until recently.

We now report an evaluation of the activity of 9-dimethylaminomethyl-10-hydroxycamptothecin (topotecan), an inhibitor of topoisomerase I, against a panel of xenografts derived from childhood ependymoma, medulloblastoma, and high-grade glioma as well as from two adult anaplastic gliomas. This agent, currently entering phase II trials in patients with a spectrum of malignancies, demonstrated considerable activity against s.c. and i.c. xenografts and may be an important new addition to the therapy of CNS tumors.

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Table 1. Activity of topotecan against s.c. xenografts

Xenograft	Derivation	Experiment	Dose (fraction of the LD_{10})[a]	Median time to 5X initial tumor volume (days)	T-C[b]	Regressions[c]
D425 Med	Medulloblastoma	1	1.0	32.8	37.3*	9/9 (9)*
		2	1.0	33.1	26.8*	6/7 (5)*
D528 EP	Ependymoma	1	1.0	82.2	55.7*	7/7 (6)*
D612 EP	Ependymoma	1	1.0	49.9	26.2*	7/7 (4)*
		2	1.0	49.7	36.1*	10/10 (8)*
D-456 MG	Childhood GBM	1	1.0	23.3	26.2*	8/8 (6)*
		2	1.0	24.3	24.4*	9/9 (6)*
D-212 MG	Childhood GBM	1	1.0	55.1	38.1*	6/7 (1)*
D-54 MG	Adult AA	1	1.0	11.4	6.3*	0/10 (0)
		2	1.0	13.5	9.9*	2/10 (0)
D-245 MG	Adult GBM	1	1.0	27.6	13.1*	8/9 (0)*
		2	1.0	27.5	16.5*	5/8 (0)*

GBM, Glioblastoma multiforme; AA, anaplastic astrocytoma

[a] Topotecan was given via daily i.p. injection at the indicated fraction of the LD_{10} on days 1–5 and 8–12 at a volume of 90 ml/m^2

[b] Growth delay in days, defined as the difference between the median time for tumors in treated (T) and control (C) animals to reach 5 times the volume recorded at initiation of treatment

[c] Defined as a decrease in tumor volume over two successive measurements. Figures in parentheses represent the number of complete regressions

* Value is statistically significant ($P<0.01$)

Materials and methods

Animals. Male or female athymic BALB/c mice (*nu/nu* genotype, 6 weeks or older) were used for all studies as described previously [5].

Xenografts. A panel of human CNS tumor-derived xenografts was used for all studies. D425 Med was derived from medulloblastomas as described previously [3]. D-212 MG and D-456 MG were derived from childhood glioblastomas multiforme as described previously [2, 13]. D528 EP and D612 EP were derived from posterior fossa ependymomas in children aged 2 and 3 years, respectively. D-245 MG was derived from an adult glioblastoma multiforme as described previously [18]. D-54 MG is the Duke University subline of A-172 established by Giard et al. [14].

Drugs. Topotecan was given once a day via i.p. injection in 0.9% saline at a volume of 90 ml/m^2. Topotecan was provided in all studies by Dr. Randall K. Johnson, SmithKline Beecham.

Dose selection studies. The dose of topotecan lethal to 10% of treated animals (LD_{10}) on a day 1–5 and 8–12 schedule was defined by log-probit analysis of cohorts of 10–20 mice treated at doses ranging between 1 and 5 mg/kg as described previously [21].

Xenograft transplantation. Subcutaneous tumor transplantation into the right flank was performed as described previously with inoculation volumes of 30 µl [9]. Intracranial tumor transplantation into the right cerebrum was performed with inoculation volumes of 5 µl using a 17-gauge needle equipped with a sleeve allowing 4.5-mm penetration as described previously [9].

Tumor measurements. Subcutaneous tumors were measured every 3–4 days with vernier calipers (Scientific Products, McGraw, Ill.). The tumor volume was calculated according to the following formula: (width)^2 ×(length)/2.

Treatment regimen. Following s.c. injection of tumor homogenate and attainment of a median tumor volume exceeding 200 mm^3, groups of 8–10 randomly selected mice were started on treatment with topotecan (doses, 0.25–1.0 LD_{10}) or 0.9% saline.

Following i.c. injection of tumor homogenate, groups of 8–10 randomly selected mice were started on treatment on the day that represented 50% of the time in days between the initial tumor inoculation and the first death, as previously defined in i.c. tumor-bearing mice receiving no therapy.

Assessment of response. The response of s.c. xenografts was assessed by delay in tumor growth and by tumor regressions. Growth delay, expressed as T–C, is defined as the difference in days between the median time for the tumors of treated (T) and control (C) animals to reach a volume 5 times that recorded at the time of original treatment. Tumor regression is defined as a decrease in tumor volume over two successive measurements. Statistical analysis was performed using the Wilcoxon rank-order test for growth delay and Fisher's exact test for tumor regressions as described previously [9].

The response of i.c. xenografts was assessed by comparing the median survival time between treated and control groups. Statistical analysis was performed using the Wilcoxon rank-order test.

Results

Dose selection studies

The LD_{10} for topotecan given as a single daily i.p. injection on days 1–5 and 8–12 was 1.9 mg/kg.

Subcutaneous xenograft therapy

Toxicity. Among the 136 animals treated, 6 deaths attributable to drug toxicity occurred. The median nadir weight loss was 4.5% ± 1.3% (± SD).

Activity. Topotecan was active in the therapy of all xenografts treated, with statistically significant ($P<0.01$) growth delays ranging between 6.3 days in D-54 MG and 55.7 days in D528 EP (Table 1). Topotecan produced statistically significant ($P<0.01$) tumor regressions in D425 Med, D-456 MG, D-245 MG, D528 EP, and D612 EP but

Table 2. Activity of topotecan against i.c. xenografts

Xenograft	Derivation	Dose (fraction of the LD_{10})[a]	Day of treatment[b]	Median day of death		Increase in median survival[c] (%)
				Control	Treated	
D-456 MG	Childhood GBM	1.0	18	38.5	49.5	39.1*
D-54 MG	Adult AA	1.0	9	23	32	28.7*

GBM, Glioblastoma multiforme; AA, anaplastic astrocytoma

[a] Topotecan was given via daily i.p. injection at the indicated fraction of the LD_{10} on days 1–5 and 8–12 at a volume of 90 ml/m^2

[b] This day represents 50% of the time in days between tumor inoculation and the first death in a cohort of 8–10 i.c. tumor-bearing mice receiving 0.9% saline alone

[c] Calculated as (median day of death of drug-treated mice) minus (median day of death of saline-treated mice) divided by median day of death of saline-treated mice

* Value is statistically significant ($P < 0.01$)

not in D-212 MG or D-54 MG (Table 1). No tumor regression was seen in any animal receiving 0.9% saline.

Intracranial xenograft therapy

Toxicity. Among the 20 animals treated, 2 deaths attributable to drug toxicity occurred.

Activity. Topotecan produced statistically significant ($P < 0.01$) increases in median survival (IMS) in both xenografts studied, yielding IMS values of 28.6% in D-456 MG and 39% in D-54 MG (Table 2).

Discussion

The failure of chemotherapy to enhance substantially the treatment of brain tumors has frequently been ascribed to the restricted access of drugs to the i.c. site secondary to the blood-brain barrier. This oversimplification has served only to delay identification of new active agents by causing studies to be focused exclusively on compounds with physiochemical properties deemed critical for passage into the brain. The fallacy of this approach has been discussed, as has the strong recommendation to define active agents first and subsequently to attempt to maximize their delivery to the brain [8]. Topotecan was chosen for evaluation in a heterogeneous panel of CNS tumor-derived xenografts due to its prodigious activity against childhood rhabdomyosarcoma xenografts and adult colon carcinoma xenografts.

Alkylating agents – including cyclophosphamide, melphalan, and cisplatin – are the only agents active in the treatment of malignant CNS tumors, and development of resistance to them leaves few chemotherapeutic options. In this study, topotecan was active against xenografts derived from medulloblastoma, ependymoma, childhood high-grade glioma, and adult high-grade glioma growing s.c. in the flanks of athymic mice, a site displaying no restriction in drug permeability. This evidence of intrinsic tumor cell sensitivity to topotecan was followed by studies evaluating drug delivery to the brain with treatment of i.c. xenografts. Topotecan produced statistically significant prolongation of survival in mice bearing i.c. xenografts, indicating that this agent achieves pharmacologically active levels in tumors growing in the brain, despite physiochemical properties conventionally deemed inadequate for treatment of i.c. tumors.

The spectrum of agents active in the therapy of CNS tumors is not increasing rapidly, with the most recent studies confirming the failure of one or another new agent. Medulloblastoma and ependymoma are sensitive to cyclophosphamide, vincristine, and cisplatin [7], and phase III studies in progress, or soon to open, will determine if these agents can increase the survival of children newly diagnosed with these tumors. A pressing need for additional active agents remains, particularly but not exclusively for children who fail these agents and who have few, if any, meaningful therapeutic options. The need for agents that are active against childhood or adult high-grade glioma is even more pressing, with virtually no chemotherapeutic regimen being effective in substantially increasing the survival of patients with these tumors (with the possible exception of CCNU-vincristine-prednisone in the treatment of children with high-grade gliomas [22]).

By virtue of its prodigious activity against a heterogeneous panel of s.c. and i.c. CNS tumor-derived xenografts, topotecan warrants evaluation in phase II trials. These trials are in progress, but, reflecting the uncertainty in the optimal dosing schedule, they include 24-h continuous-infusion, 72-h continuous-infusion, and 1-h bolus (for 5 consecutive days) regimens. Nevertheless, early data support activity for each regimen, with histiotypic specificity not yet being discernible. The forthcoming development of a formulation suitable for oral administration will allow evaluation of the most promising preclinical regimen, extended once-a-day therapy [16]. Topotecan may be an important new agent in the treatment of CNS tumors.

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