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ORIGINAL ARTICLE

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Therapeutic activity of 7-[(2-trimethylsilyl)ethyl)]-20 (S)-camptothecin against central nervous system tumor-derived xenografts in athymic mice

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Abstract *Purpose*: Camptothecins have emerged as an important new class of antitumor drugs. Camptothecin derivatives such as CPT-11 and topotecan are commercially available and approved for the treatment of colorectal (CPT-11) and ovarian and small-cell lung cancer (topotecan). This study was designed to test the efficacy of karenitecin, a novel highly lipophilic camptothecin derivative, against a panel of human tumor xenografts derived from adult and pediatric central nervous system malignancies growing in athymic nude mice. *Methods*: Xenografts evaluated were derived from childhood high-grade gliomas (D-212 MG, D-456 MG), adult high-grade gliomas (D-54 MG, D-245 MG), medulloblastomas (D-341 MED, D-487 MED), and ependymomas (D-528 EP, D-612 EP), as well as sublines with demonstrated resistance to procarbazine (D-245 MG (PR)) and busulfan (D-456 (BR)). In replicate experiments, karenitecin was given at 1.0 mg/kg per dose via intraperitoneal injection for a period of 10 consecutive days, which is the dosage lethal to 10% of treated animals. *Results*: Karenitecin produced statistically significant ($P \leq 0.001$) growth delays in all subcutaneous xenografts tested, including the sublines resistant to procarbazine and busulfan. Growth delays ranged from 12.1 days in D-456 MG (BR) to 90+ days in D-212 MG and D-341 MED. Karenitecin also produced statistically significant ($P \leq 0.001$) increases in survival of animals bearing D-341 MED intracranial xenografts (69% increase) and those bearing D-456 MG xenografts (62% increase). *Conclusion*: These preclinical studies confirm that karenitecin is active against human central nervous system xenografts and should undergo clinical evaluation in patients with malignant central nervous system tumors.

Keywords Karenitecin · Central nervous system malignancies · Camptothecin derivatives · Human tumor xenografts

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Introduction

Malignant tumors arising in the central nervous system are conventionally treated with local therapy, specifically surgery and radiotherapy. Unfortunately, this treatment fails to control malignant tumor recurrence at the primary site and does not affect tumor cells that have invariably spread throughout the brain at diagnosis [3, 12]. Therefore, effective systemic drug therapy is critically needed to increase survival of children and adults with these neoplasms.

Despite the marked potential for new therapeutic strategies such as gene therapy or antitumor vaccines to more effectively treat human malignancies including primary brain tumors, these approaches remain an unfulfilled hope. Many technological problems must be resolved, including delivery and target specificity before meaningful antitumor activity is seen in the clinic. Accordingly chemotherapy remains the primary modality chosen to augment the activity of surgery and radiotherapy. Unfortunately, resistance to virtually all of the commercially available chemotherapeutic agents severely limits the benefits of chemotherapy in treating human malignancies providing an impetus for efforts to identify new agents.

Camptothecins have emerged over the last decade as an important new class of antineoplastic agents. Severalagents in this class, including topotecan and irinotecan (CPT-11) are commercially available and have been shown to have antitumor activity against malignant gliomas [8, 9]. These agents contain a 20(S)

α-hydroxy-δ-lactone E-ring moiety that undergoes base-mediated hydrolysis at physiologic pH, with opening of the lactone E-ring to an inactive carboxylate form. It has been established that the lactone E-ring of the camptothecin molecule is responsible for antitumor activity and that the carboxylate form is inactive in terms of antitumor effects. All camptothecins that have undergone development in the clinic including CPT-11, 9-amino camptothecin, 9-nitro camptothecin, topotecan and DX8915f show relatively low concentrations of lactone E-ring in patient plasma – ranging from 0.5% to 20%. Unfortunately, the concentration of the inactive 20(S) lactone species of these agents in human plasma is 20% or less of the total drug concentration, and represents a major limitation of these drugs [2].

An additional problem adversely affecting the activity of CPT-11 is the large interpatient variability of prodrug conversion to SN38, which is further compounded by additional interpatient variability of SN38 glucuronide levels. Following administration of CPT-11, SN38 glucuronide accumulates at a log higher level than free SN38 in patient plasma and is essentially inactive as an antitumor agent. Glucuronidation of SN38 and enhanced clearance of CPT-11, SN38 and SN38 glucuronide are significantly increased by the administration of anticonvulsants and dexamethasone, cytochrome p450 agents which are commonly employed in patients with brain tumors; this is important because less-active SN38 is available in plasma [9, 10]. Drugs that have higher lactone concentrations and reduced metabolism would be highly desirable to overcome these limitations.

Karenitecin is a novel highly lipophilic camptothecin with marked activity against a broad spectrum of murine and human tumor cell lines and xenografts including glioma, prostate, breast, colon, ovarian, melanoma, lung and other tumors [11, 12]. In extensive preclinical studies karenitecin has been shown not to be susceptible to common tumor-mediated drug resistance mechanisms, including MDR/MRP/LRP or BCRP [13]. A phase 1 trial of the karenitecin has shown that the drug has a markedly higher concentration of the lactone ring (average of 89% lactone form of the AUC at the maximum tolerated dose), does not undergo glucuronidation, and has a longer plasma half-life (average of 17 h) than other camptothecins in patients' plasma (data not shown).

In the current studies, karenitecin was tested against a panel of human tumor xenografts in athymic mice derived from adult and pediatric central nervous system malignancies. Tumors tested included childhood high-grade gliomas (D-212 MG, D-456 MG), adult high-grade gliomas (D-54 MG, D-245 MG), medulloblastomas (D-341 MED, D-487 MED), and ependymomas (D-528 EP, D-612 EP), as well as sublines of these xenografts with laboratory-generated resistance to busulfan (D-456 (BR)), and procarbazine (D-245 MG (PR)). Administration of karenitecin produced statistically significant growth delays in all subcutaneous xenografts tested, including those resistant to busulfan and procarbazine. Tumor regression was evident in every xenograft line. Statistically significant increases in survival were also demonstrated in animals with the two intracranial xenografts treated.

Materials and methods

Animals

Male and female athymic BALB/c mice (*nu*/*nu* genotype, 6 weeks of age or older) were used for all studies as previously described [1].

Xenografts

A panel of 11 human CNS tumor-derived xenografts were used for all in vivo studies and were maintained as previously described [5].

Xenograft transplantation

Tumors were removed from the host under sterile conditions in a laminar flow containment hood. Tumor was segmented and placed into a modified tissue press and passed through a bi-layered mesh cytosieve to form a tumor homogenate. This homogenate was then passed through a 19-gauge needle before being placed into a 250-μl Hamilton syringe dispenser. This was used to inoculate the right flank of animals with 50 μl tumor homogenate as previously described [1].

Intracranial (i.c.) tumor transplantation into the right cerebrum was performed as described previously with inoculation volumes of 10 μl [4].

Tumor measurements

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

Xenograft therapy

Karenitecin (BioNumerik Pharmaceuticals, San Antonio, Texas) was given to mice via intraperitoneal injection at 1.0 mg/kg per dose daily for 10 consecutive days, which represents the dose lethal to 10% of treated animals. For the subcutaneous (s.c.) studies, groups of randomly selected mice were treated when the tumor volume was within the range 100–500 mm^3 and compared with control animals receiving no treatment. For the i.c. studies, groups of ten randomly selected animals were treated using the same regimen of karenitecin as described for the s.c studies on the day which represented 50% of the time elapsing between the initial tumor inoculation and the median day of death as previously determined in the 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 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 analysis was 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 [4]. 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 [4].

Results

Toxicity

Among the 220 treated animals, 19 deaths were attributable to drug toxicity. The median weight loss nadir was 7.4% among the surviving animals. No neurologic toxicity, including seizure activity, was noted.

Subcutaneous xenograft therapy

Karenitecin was active against all tumor lines tested, including those resistant to procarbazine and busulfan (Table 1). Growth delays ranged from 12.1 days in D-456 MG (BR) to 90+ days in D-212 MG and D-341 MED. All values were statistically significant ($P \leq 0.001$). Tumor regressions were seen in all xenografts.

Intracranial xenograft therapy

Karenitecin produced statistically significant ($P \leq 0.001$) increases in the median survival of mice bearing i.c. tumor xenografts (69% increase in D-341 MED and 62% increase in D-456 MG; Table 2). All mice displayed gross evidence of i.c. tumor at time of death.

Discussion

The treatment of malignant brain tumors continues to be a therapeutic challenge, with surgery and radiotherapy providing only limited benefits [3, 12, 14]. Drug resistance, common at the time of diagnosis of malignant glioma, almost invariably develops during therapy with consequent tumor growth and patient death [3]. Development of new chemotherapeutic strategies is needed to improve the outcome for patients with malignant brain tumors.

Camptothecins are emerging as active chemotherapeutic agents for a spectrum of human malignancies [16]. These agents, shown to produce antineoplastic activity by inhibition of topoisomerase I, contain a 20(S) α-hydroxy-δ-lactone E-ring moiety which undergoes pH-mediated hydrolysis to form an inactive carboxylate species. Topotecan and CPT-11 display antiglioma activity [8, 9] while achieving a lactone ring concentration of only 20% or less of the total drug concentration. In addition, these commercially available drugs undergo unfavorable metabolism, including glucuronidation, oxidation and other transformations, which can limit their efficacy. It has been observed that the administration of anticonvulsants and dexamethasone can enhance the clearance and potentially the unfavorable metabolism of CPT-11, thereby limiting effective plasma levels of active drug [9, 10].

Newer camptothecins have been engineered to increase the concentration of lactone ring in human plasma and to reduce unfavorable metabolism/clearance with the hope of increasing antineoplastic activity. Karenitecin is a new rationally engineered camptothecin designed to achieve high lactone species concentrations

Table 1 Effect of karenitecin on growth of subcutaneous human CNS xenografts in mice. In replicate studies, karenitecin was administered via intraperitoneal injection at 1.0 mg/kg per dose in normal saline for 10 consecutive days

Xenograft	Histology	T–C (days)[a]	Regressions[b]
D-212 MG	Childhood high-grade glioma	90+	10/10
		90+	10/10
D-456 MG	Childhood high-grade glioma	45.9	8/10
		44.9	7/10
D-456 MG (BR)	Childhood high-grade glioma, busulfan-resistant	22.0	8/8
		12.1	4/9
D-54 MG	Adult high-grade glioma	21.7	8/10
		16.1	5/8
D-245 MG	Adult high-grade glioma	32.2	10/10
		24.3	7/10
D-245 MG (PR)	Adult high-grade glioma, procarbazine-resistant	57.5	8/8
		53.2	10/10
D-341 MED	Medulloblastoma	90+	10/10
		90+	8/10
D-487 MED	Medulloblastoma	20.0	7/7
		14.0	3/10
D-528 EP	Ependymoma	64.6	9/9
		65.3	10/10
D-612 EP	Ependymoma	34.9	7/7
		36.3	10/10

[a]Growth delay in days is 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 studies are statistically significant ($P \leq 0.001$) compared with controls

[b]Regression is defined as a decrease in tumor volume over two successive measurements

Table 2 Effect of karenitecin treatment on survival of mice bearing intracranial human CNS xenografts. Karenitecin was administered via intraperitoneal injection at 1.0 mg/kg per dose in normal saline for a period of 10 consecutive days

Xenograft	Histology	Median day of death		Increase in median survival (%)[a]
		Control	Treated	
D-341 MED	Medulloblastoma	22.5	38	69
D-456 MG	Childhood high-grade glioma	34	55	62

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

in human plasma and to avoid glucuronidation relative to commercially available camptothecins. This pharmacologic advantage, coupled with marked broad-spectrum antineoplastic activity against an extensive range of human and murine tumors, provided the rationale for the current studies [11, 12]. The current results clearly show marked activity of karenitecin against a broad spectrum of xenografts derived from CNS tumors. The tumor growth delays reported here indicate antitumor activity commensurate with the most effective chemotherapeutic agents previously evaluated in our CNS tumor model in nude mice, specifically temozolomide [6] and CPT-11 [10]. Both of these agents have subsequently been shown to demonstrate antiglioma activity in the clinic [7, 9]. Based on our results, we believe that evaluation of karenitecin in children and adults with recurrent malignant brain tumors is warranted.

In the future it will be important to determine whether karenitecin's favorable preclinical antitumor activity and the encouraging phase 1 clinical pharmacology and safety profile, along with substantially higher plasma levels of lactone ring species, will result in greater antitumor efficacy for brain tumor patients. The initial phase 1 trial of karenitecin has demonstrated that the drug appears to be very well tolerated by patients with minimal diarrhea, nausea and vomiting [15]. The dose-limiting toxicity which occurred more frequently in patients treated above the recommended phase 2 dose is reversible myelosuppression in the form of neutropenia and thrombocytopenia. It is notable that, in the phase 1 trial, 7 out of 15 evaluable patients who had received an average of three prior chemotherapy regimens prior to study entry and included four patients who failed prior CPT-11 therapy, exhibited prolonged stable disease.

The promising safety and efficacy profile for karenitecin will be further defined in histology-specific phase 2 trials. An additional consideration for patients with brain tumors who are on the anticonvulsants dilantin, tegretol, or phenobarbital, is the dramatically enhanced metabolism and excretion of CPT-11 or topotecan metabolites [9]. This substantial perturbation of CPT-11 metabolism and elimination has made it extremely difficult to dose patients on anticonvulsants and dexamethasone. Karenitecin does not undergo glucuronidation and appears to have limited metabolism. Anticonvulsants and dexamethasone may have reduced effects on the metabolism and elimination karenitecin, which would make the drug potentially superior in this setting.

A phase 2 trial of karenitecin will be initiated in the near future for adults with recurrent malignant glioma to rigorously evaluate its safety, efficacy and drug-mediated alterations in drug metabolism and clearance.

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