Novel Nanoliposomal CPT-11 Infused by Convection-Enhanced Delivery in Intracranial Tumors: Pharmacology and Efficacy

Charles O. Noble,[1] Michal T. Krauze,[2] Daryl C. Drummond,[3] Yoji Yamashita,[2] Ryuta Saito,[2] Mitchel S. Berger,[2] Dmitri B. Kirpotin,[3] Krystof S. Bankiewicz,[2] and John W. Park[1]

[1]Division of Hematology/Oncology and [2]Department of Neurological Surgery, Brain Tumor Research Center, University of California at San Francisco, San Francisco, California and [3]Hermes Biosciences, Inc., South San Francisco, California

Abstract

We hypothesized that combining convection-enhanced delivery (CED) with a novel, highly stable nanoparticle/liposome containing CPT-11 (nanoliposomal CPT-11) would provide a dual drug delivery strategy for brain tumor treatment. Following CED in rat brains, tissue retention of nanoliposomal CPT-11 was greatly prolonged, with >20% injected dose remaining at 12 days for all doses. Tissue residence was dose dependent, with doses of 60 μg (3 mg/mL), 0.8 mg (40 mg/mL), and 1.6 mg (80 mg/mL) resulting in tissue half-life ($t_{1/2}$) of 6.7, 10.7, and 19.7 days, respectively. In contrast, CED of free CPT-11 resulted in rapid drug clearance (tissue $t_{1/2}$ = 0.3 day). At equivalent CED doses, nanoliposomal CPT-11 increased area under the time-concentration curve by 25-fold and tissue $t_{1/2}$ by 22-fold over free CPT-11; CED in intracranial U87 glioma xenografts showed even longer tumor retention (tissue $t_{1/2}$ = 43 days). Plasma levels were undetectable following CED of nanoliposomal CPT-11. Importantly, prolonged exposure to nanoliposomal CPT-11 resulted in no measurable central nervous system (CNS) toxicity at any dose tested (0.06-1.6 mg/rat), whereas CED of free CPT-11 induced severe CNS toxicity at 0.4 mg/rat. In the intracranial U87 glioma xenograft model, a single CED infusion of nanoliposomal CPT-11 at 1.6 mg resulted in significantly improved median survival (>100 days) compared with CED of control liposomes (19.5 days; $P = 4.9 \times 10^{-5}$) or free drug (28.5 days; P = 0.011). We conclude that CED of nanoliposomal CPT-11 greatly prolonged tissue residence while also substantially reducing toxicity, resulting in a highly effective treatment strategy in preclinical brain tumor models. (Cancer Res 2006; 66(5): 2801-6)

Introduction

Outcomes for brain tumor patients, particularly those with high-grade gliomas, remain suboptimal and highlight the need for novel therapeutic approaches. Because restricted access is one of the hallmarks of these tumors, strategies for improving drug delivery have attracted much interest. These strategies include regional administration approaches within the central nervous system (CNS) as well as particle-based carriers of drugs.

Convection-enhanced delivery (CED) is a local-regional drug delivery technique that uses a pressure-driven bulk-flow process to distribute agents, including macromolecules, to clinically relevant volumes of solid tissues (1, 2). CED can be used to circumvent the blood-brain barrier, which is a considerable obstacle for many systemically applied drugs (3, 4). CED represents a promising approach to treat various CNS diseases, including brain tumors, which cannot be controlled by local treatment and are poorly responsive to systemic treatment. Compared with routes of administration dependent on diffusion from the injection/implantation site, CED shows a greater volume of distribution and is designed to direct a drug to specific target volumes (5–8).

Liposomes are nano- or microscale carriers typically consisting of a phospholipid membrane shell surrounding a hollow core that can be used to encapsulate small molecules. Liposomal anthracyclines represent the first successful examples of nanoparticle-based anticancer treatment, including marketed agents pegylated liposomal doxorubicin (Doxil, Alza Pharmaceuticals, Inc., Mountain View, CA; Caelyx, Schering-Plough, Inc., Kenilworth, NJ) and liposomal daunorubicin (Daunoxome, Gilead, Inc., Foster City, CA; refs. 9–14). To encapsulate other drugs, we have recently developed a novel intraliposomal drug loading and stabilization technology: poly(anionic) polyols were used to generate new liposomal drugs with unusual drug stability and favorable preclinical pharmacokinetics (15–17). One of these new agents, nanoparticle/liposome containing CPT-11 (nanoliposomal CPT-11; refs. 18–20), encapsulates the camptothecin derivative and topoisomerase I inhibitor CPT-11/irinotecan (Camptosar, Pfizer, New York, NY). Although highly active against many cancer types, CPT-11 displays complex pharmacology characterized by diverse biochemical transformations and potential toxicities (21, 22). In particular, CPT-11 requires conversion to SN-38 for optimal activity yet must avoid inactivation via simple hydrolysis of the requisite lactone configuration to an inactive carboxylate. Furthermore, hepatic conversion to SN-38 leads to biliary excretion of this potent metabolite and resulting gastrointestinal toxicity. In principle, the pharmacologic profile of CPT-11 can be improved by tumor-directed drug delivery, including liposomal encapsulation.

We hypothesized that a combined drug delivery approach featuring nanoliposomal CPT-11 given by CED is feasible and therapeutically advantageous. In previous studies, we showed that CED can be used to achieve extensive distribution of liposomes in rodent brains, orthotopic brain tumor xenografts (23, 24), and monkey brains (25–27). We now report the pharmacology and efficacy of a novel nanoparticle/liposome-based drug given by CED in preclinical brain tumor models.

Materials and Methods

Nanoparticle/liposome constructs. Lipids included 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (PEG-DSPE; Avanti Polar Lipids, Inc., Alabaster, AL), and cholesterol (Calbiochem, San Diego, CA). Small unilamellar liposomes were composed of DSPC, cholesterol, and

Note: C.O. Noble and M.T. Krauze contributed equally to this work.

Requests for reprints: John W. Park, University of California San Francisco, Division of Hematology-Oncology, 1600 Divisidaro Street 2nd Floor, San Francisco, CA 94115. Phone: 415-502-3844; Fax: 415-353-9571; E-mail: jpark@cc.ucsf.edu.

©2006 American Association for Cancer Research.

doi:10.1158/0008-5472.CAN-05-3535

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/5/2801/2559783/2801.pdf by guest on 05 February 2023

PEG-DSPE at a molar ratio of 3:2:0.015. Liposomes were prepared by dissolving all lipids in chloroform/methanol (9:1, v/v) and removing solvent by rotary evaporation to form a dried lipid foam. After hydration, the lipid suspension was briefly vacuumized on a rotary evaporator to remove any trace of organic solvents. Unilamellar liposomes were formed by extrusion at 60°C using gas-pressure thermostatted barrel extruder (Lipex Biomembranes, Vancouver, British Columbia, Canada) through polycarbonate membranes (Whatman Nucleopore, Clifton, NJ) having a pore size of 200 nm (6 times) and 100 nm (12 times) and yielding a final diameter of 96 to 101 nm as determined by light scattering (N4Plus particle size analyzer, Beckman Coulter, Fullerton, CA). *N,N′*-bis-octadecyl-4,4,4′,4′-tetramethylindacarbocyanin iodide [DiIC_{18}(3); Molecular Probes, Inc., Eugene, OR] was included for fluorescent labeling.

Drug loading. To prepare triethylammonium sucrose octasulfate (TEA-SOS) as a drug-trapping agent, 0.3 mol/L sodium sucrose octasulfate (Toronto Research Chemicals, Inc., North York, Ontario, Canada) was applied to a column containing Dowex 50W-8X-200 cation exchange resin (equilibrated with 3 mol/L HCl) to convert the sodium salt of sucrose octasulfate to free acid form. Sucrose octasulfuric acid was eluted from the column with double-distilled water using an inline conductivity detector. The solution was then neutralized with neat triethylamine and diluted to a concentration corresponding to 0.65 mol/L triethylammonium with an osmolality of 480 to 530 mmol/kg (pH 5.5-6.0). Residual sodium was determined by potentiometry using a sodium-sensitive glass electrode. For TEA-SOS-containing liposomes, dried lipids were hydrated in 81 mmol/L aqueous TEA-SOS solution (0.65 mol/L triethylamine) at 60°C, and the hydrated lipid suspension was subjected to eight cycles of freezing (−80°C) and thawing (60°C). Extraliposomal TEA-SOS was removed by size exclusion chromatography on Sepharose CL-4B.

CPT-11 (a kind gift of PharmaEngine, Inc., Taipei, Taiwan) was loaded into TEA-SOS-containing liposomes by addition of a 15 mg/mL solution of CPT-11/HCl to a final drug-to-lipid ratio of 500 g CPT-11/mol phospholipid (for 0.06-0.8 mg/rat dose levels) or 800 g CPT-11/mol phospholipid (for 1.6 mg/rat dose level), with incubation of the drug-liposome mixture at 60°C (pH 6.0) for 45 minutes followed by quenching on ice for 15 minutes. Unencapsulated CPT-11 was removed by Sephadex G75 size exclusion chromatography, and the drug-loaded liposomes were stored at 4°C until use. The resulting nanoliposomal CPT-11 was concentrated on a stirred cell concentrator containing a regenerated cellulose 1×10^5 NMWL membrane (Amicon, Millipore Corp., Billerica, MA) and sterilized by passage through 0.2-μm PES syringe filter. CPT-11 concentration was determined by measuring absorbance at 375 nm of a solubilized sample. Briefly, 0.1 mL of an aqueous portion of the sample containing nanoliposomal CPT-11 or standards was added to 0.9 mL of a solution containing 72 vol % methanol, 18 vol % of 0.1 mol/L phosphoric acid, and 10 vol % chloroform. Phospholipid was measured using blue phosphomolybdate-based spectrophotometric assay (28).

Animal models. Toxicity studies used healthy male Sprague-Dawley rats weighing ~250 g (Charles-River Laboratories, Wilmington, MA). For xenograft studies, congenitally athymic, male, nude rats (*rnu/rnu*, homozygous) weighing ~150 to 200 g (National Cancer Institute Animal Production Program, Frederick, MD) were housed under aseptic conditions, which included filtered air and sterilized food, water, bedding, and cages. For the intracranial xenograft tumor model, U87 glioblastoma cells (Brain Tumor Research Center Tissue Bank, University of California at San Francisco, San Francisco, CA) were harvested by trypsinization, washed once with HBSS without Ca^{2+} and Mg^{2+}, and resuspended in HBSS for implantation. Cells (5×10^5) in 10 μL HBSS were implanted into the striatal region of athymic rat brains as follows: under deep isoflurane anesthesia, rats were placed in a small-animal stereotaxic frame (David Kopf Instrument, Tujunga, CA). A sagittal incision was made to expose the cranium and followed by a burr hole in the skull at 0.5 mm anterior and 3 mm lateral from the bregma using a small dental drill. Cell suspension (5 μL) was injected over 2 minutes at a depth of 4.5 mm from the brain surface; after a 2-minute wait, another 5 μL were injected over 2 minutes at a depth of 4 mm, and after a final 2-minute wait, the needle was removed and the wound was sutured.

Convection-enhanced delivery. CED of free CPT-11 or nanoliposomal CPT-11 was done using a volume of 20 μL as described (24, 29). Briefly, the infusion system consisted of a fused-silica needle cannula that was connected to a loading line (containing the therapeutic agent) and an olive oil infusion line. A 1-mL syringe (filled with oil) mounted onto a microinfusion pump (BeeHive, Bioanalytical Systems, West Lafayette, IN) regulated the flow of fluid through the system. Based on chosen coordinates, needle cannula was mounted onto stereotactic holders and guided to targeted region of the brain through burr holes made in the skull. The following ascending infusion rates were applied to achieve the 20-μL total infusion volume: 0.2 μL/min (15 minutes) + 0.5 μL/min (10 minutes) + 0.8 μL/min (15 minutes).

Tissue pharmacokinetics. Rats were given a single 20-μL infusion by CED of free or nanoliposomal CPT-11, and the animal was sacrificed at prescribed times. The appropriate brain hemisphere was perfused with PBS, surgically removed, and frozen. Either the tissue was ground under liquid nitrogen or water was added to the tissue at a 50% (w/w) ratio, and the tissue was homogenized using a mechanical homogenizer in an ice bath. The homogenates (0.1 mL) were extracted as the lactone form of CPT-11 and SN-38 with 0.4 mL of an acidic methanol solution (20% 0.1 mol/L phosphoric acid/80% methanol) by vortexing for 10 seconds twice and centrifugation at 13,000 rpm for 10 minutes, and the supernatants were transferred to autosampler vials for high-pressure liquid chromatography (HPLC) analysis. Blank homogenates were added to CPT-11 and SN-38 to estimate extraction efficiency. Analysis was conducted on a Dionex HPLC system using a C_{18} reverse-phase silica column preceded by a Supelco C_{18} guard column. A sample injection volume of 50 μL was used, and the column was eluted isocratically at a flow rate of 1.0 mL/min with a mobile phase consisting of 3% by volume of aqueous (pH 5.5) triethylammonium acetate and acetonitrile (73:27). CPT-11 and SN-38 were typically eluted in 5.1 and 9.8 minutes, respectively, and both were detected by fluorescence at 420 nm (365 nm excitation).

Tissue pharmacokinetics was fit to a monoexponential decay equation using the trend analysis of Microsoft Excel (Microsoft Corp., Redmond, WA). Pharmacokinetic variables, including tissue half-life ($t_{1/2}$), clearance, mean residence time in brain or brain tumor tissue, and area under the time-concentration curve (AUC_{∞}), were all determined by noncompartmental pharmacokinetics data analysis using PK Solutions 2.0 software (Summit Research Services, Montrose, CO).

Therapy studies. To evaluate toxicity, healthy rats received a single infusion of free or nanoliposomal CPT-11 via CED. Rats were monitored daily for survival, weekly weights, and general health (alertness, grooming, feeding, excreta, skin, fur, mucous membrane conditions, ambulation, breathing, and posture). Rats were euthanized 60 days after CED treatments, and their brains were removed, fixed in 10% buffered formalin phosphate and then in 30% sucrose, and cut into sections (25 μm) for H&E staining.

To evaluate survival, rats were randomly assigned to five groups (n = 8 rats per group) and U87 tumor cells were implanted into each rat brain. Five days after tumor implantation, a single CED infusion of 20 μL was done using different treatment conditions as described in the text. Rats were evaluated for clinical and tissue toxicity as described above.

Results

Construction of nanoliposomal CPT-11 for brain tumor treatment. CPT-11 was encapsulated in small unilamellar phospholipid vesicles at extremely high concentrations not previously attainable using intraliposomal sucrose octasulfate for high capacity binding to CPT-11, combined with effective transmembrane exchange of intraliposomal triethylammonium for the drug cation (15, 16). Drug loading efficiencies were 86% to 101% of added drug. Constructs were 96 to 101 nm in diameter and achieved drug-to-lipid ratios of 691 to 812 g CPT-11/mol phospholipid, corresponding to payloads of 0.9×10^5 to 1.0×10^5 drug molecules per nanoparticle based on 8×10^5 phospholipids/nanoparticle. The resulting nanoparticle/liposome construct

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/5/2801/2559783/2801.pdf by guest on 05 February 2023

(termed nanoliposomal CPT-11 to indicate a lipid bilayer-based nanoparticle encapsulating a stably entrapped nanoscale drug complex) could be concentrated without aggregation or precipitation up to 80 mg CPT-11/mL in aqueous solution, which is ~4-fold greater than the solubility of CPT-11 as a free drug. Nanoliposomal CPT-11 showed excellent storage stability at 4°C; at 6 months, no particle size change and only 0.3% drug leakage were detected.

Tissue pharmacokinetics of nanoliposomal CPT-11 following CED in normal adult rat brains or intracranial U87 tumor xenografts. Free CPT-11 or nanoliposomal CPT-11 were given via single CED treatment into the brains of normal adult Sprague-Dawley rats, and tissue levels were determined at varying times after infusion by HPLC. Tissue retention of nanoliposomal CPT-11 was dose dependent, with doses of 60 μg (3 mg/mL), 0.8 mg (40 mg/mL), and 1.6 mg (80 mg/mL) resulting in brain tissue $t_{1/2}$s of 6.7, 10.7, and 19.7 days, respectively (Table 1). Tissue concentration versus dose was not a linear function, as even low doses (60 μg) of nanoliposomal CPT-11 resulted in substantial levels of drug: >20% injected dose (ID) remained at 12 days after infusion. In contrast, CED of free CPT-11 at the equivalent dose, which was also its highest tolerable dose tested, was cleared from the brain within 1 day (Fig. 1). Hence, at equivalent doses of nanoliposomal and free CPT-11, the AUC_∞ was increased by 25-fold for nanoliposomal CPT-11, and the tissue $t_{1/2}$ was improved from 0.3 day for free CPT-11 to 6.7 days (22-fold) for the liposomal drug. Because nanoliposomal CPT-11 could be infused at much higher doses than free CPT-11 due to its reduced toxicity (shown in the next section), nanoliposomal CPT-11 at its highest tested dose improved the AUC_∞ by 1,636-fold and tissue $t_{1/2}$ by 66-fold over that of free CPT-11 at its highest tolerable dose.

Systemic levels of CPT-11 following CED of nanoliposomal CPT-11 were measured concurrently in the plasma of healthy rats receiving 1.6 mg nanoliposomal CPT-11 (Fig. 1). Plasma CPT-11 levels were not detectable in any sample, indicating that CED abrogated significant systemic exposure.

Drug delivery to tumor tissue was evaluated in an orthotopic U87 tumor xenograft model in athymic rats, in which U87 glioma cells were implanted intracranially and allowed to grow for 10 days before treatment. In comparison with normal brain tissue, drug clearance from U87 tumors was significantly slower (Fig. 2*A*; Table 1). The tissue $t_{1/2}$ of drug (Table 1) was extended by ~4-fold (10.7 versus 43.0 days) when nanoliposomal CPT-11 was infused in the tumor xenograft model compared with normal rat brain tissue.

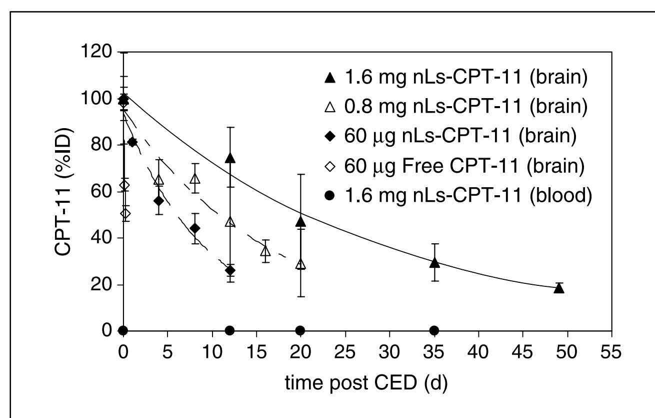

Figure 1. Tissue pharmacokinetics of free CPT-11 and nanoliposomal (nLs) CPT-11 in the normal adult rat brain and blood following single CED infusion. All values are %ID versus time after CED of 20-μL infusate. Drug concentrations were determined by HPLC assay for CPT-11/HCl. CPT-11 concentrations in blood were all below the detection limit of 1 ng/mL (~0.03%ID).

Tumor penetration of free CPT-11 given systemically (tail vein injection) in rats with intracranial U87 tumors was examined by measuring the drug in tumor tissue and plasma (Fig. 2*B*). Fifteen minutes after i.v. injection of free CPT-11 at its established maximum tolerated doses of 100 mg/kg (29.3 mg; ref. 30), drug levels in tumor were 2.7 μg/g (0.009% ID/g tissue), and at 5 hours, this had declined to 1.7 μg/g. These miniscule concentrations were much lower than the 46.2 μg/g tissue achieved 15 minutes after CED of free CPT-11 (17-fold) and, even more so, the 921 μg/g tissue after CED of 1.6 mg nanoliposomal CPT-11 (341-fold).

SN-38, the highly active metabolite of CPT-11, was also assayed in tissue and plasma samples but was undetectable in all samples. Using several different extraction methods for the detection of SN-38 by HPLC analysis (31–33), a detection limit of 1 ng/mL was established with spiked tissue recovery of >95% using an acidic methanol extraction.

Host toxicity of CED of free CPT-11 and nanoliposomal CPT-11 in rodent CNS. Free CPT-11 (60 μg or 0.4 mg) or nanoliposomal CPT-11 (0.06, 0.4, 0.8, and 1.6 mg) were given via a single 20-μL CED infusion into normal adult rat brains (Fig. 3). After sacrifice at 42 days after treatment, histologic evidence of neurotoxic injury was scored on a scale of 0 to 3+. In animals receiving CED of free CPT-11 at 60 μg, brain tissue contained evidence of minor trauma at the

Table 1. Tissue pharmacokinetics of CPT-11 formulations given by CED

	$t_{1/2}$ (d)	AUC_∞ (μg d/g)	Clearance (g/d)	Mean residence time (d)
Brain				
60 μg free CPT-11	0.3	16.4	3.6	0.4
60 μg nanoliposomal CPT-11	6.7	417	0.14	9.6
0.8 mg nanoliposomal CPT-11	10.7	13,723	0.058	15.4
1.6 mg nanoliposomal CPT-11	19.7	26,823	0.06	28.5
U87 Tumor				
0.8 mg nanoliposomal CPT-11	43.0	40,315	0.02	62.0

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/5/2801/2559783/2801.pdf by guest on 05 February 2023

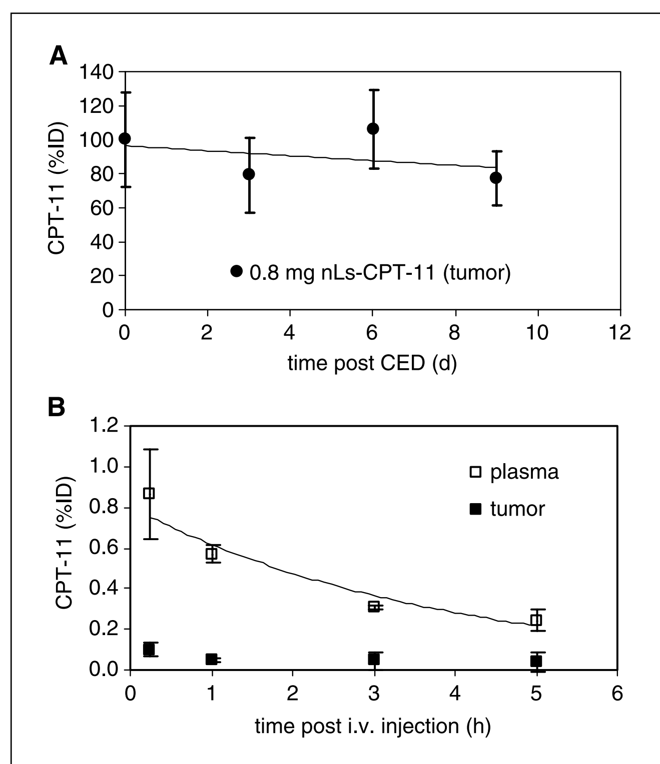

Figure 2. Tumor tissue pharmacokinetics of free CPT-11 and nanoliposomal CPT-11 in intracranial U87 tumor xenografts. Tumors were implanted and allowed to grow for 10 days before CED or i.v. treatment. *A,* nanoliposomal CPT-11, single CED treatment at 0.8 mg (20 μL). *B,* free CPT-11, following bolus i.v. injection at 100 mg/kg. Two to four rats per time point.

site of the infusion cannula (arrows) in the striatum but otherwise no apparent tissue toxicity (score = 0; Fig. 3*A*). However, all animals that received free CPT-11 at 0.4 mg/rat were observed to have extensive tissue necrosis within the CNS (score = 3+; Fig. 3*B*). In contrast, animals receiving nanoliposomal CPT-11 at all doses tested showed no evidence of CNS toxicity (score = 0 for all animals), and the only finding was minor trauma at the infusion cannula site (Fig. 3*C*).

No systemic toxicities, including weight loss or diarrhea, were observed following CED of any of the treatments (Fig. 3*D*). Furthermore, no gross neurologic or behavioral changes were noted after treatment. Indeed, no dose-limiting toxicities for nanoliposomal CPT-11 were identified up to 1.6 mg/rat, which represented the highest feasible dose. Higher doses were precluded by formulation viscosity at concentrations >80 mg/mL, although this was 4-fold greater than the solubility of free CPT-11. Taken together, these data indicated that nanoliposomal CPT-11 greatly extended the tissue tolerance and maximum tolerated doses of the drug; whereas the highest tolerable dose of free CPT-11 was 60 μg/rat, that for nanoliposomal CPT-11 was at least 1.6 mg/rat.

Efficacy of CED of free CPT-11 and nanoliposomal CPT-11. The antitumor efficacy of nanoliposomal CPT-11 (0.06, 0.8, and 1.6 mg/rat) and free CPT-11 (at the highest tolerable dose tested, 0.06 mg/rat) was evaluated following single CED infusion in the intracranial U87 tumor xenograft model. The control group received a CED infusion of "empty" liposomes of the same lipid composition as nanoliposomal CPT-11 but without any encapsulated drug and labeled with the lipophilic fluorescent marker, $DiIC_{18}(3)$. As shown in Fig. 4, all animals in the control group expired due to tumor progression by day 22, and mean survival was only 20 days (median, 19.5 days). Treatment with free CPT-11 showed a slight improvement in survival, although all animals still expired by day 30 and mean survival was 28 days (median, 28.5 days). At the equivalent dose of 0.06 mg/rat, treatment with nanoliposomal CPT-11 resulted in mean survival of 36 days (median, 30 days) and one of eight rats surviving beyond 100 days; this suggested a trend toward superiority for nanoliposomal CPT-11 over free drug ($P = 0.09$, pairwise comparison). Treatment with nanoliposomal CPT-11 at 0.8 mg/rat resulted in 50% of the animals surviving beyond day 100 and mean survival of 71 days (median, 78 days). Animals treated with nanoliposomal CPT-11 at 1.6 mg/rat showed excellent survival, with five of eight rats surviving beyond day 100 and mean survival of 83 days (median, >100 days). Overall, CED infusion of nanoliposomal CPT-11 produced greatly enhanced survival compared with CED of free CPT-11 (hazard ratio = 0.39; $P = 0.01$). The improved survival associated with nanoliposomal CPT-11 treatment was dose dependent, with risk of death versus control of 90%, 24%, and 5.8% for the animals receiving the 0.06, 0.8, and 1.6 mg/rat treatments, respectively ($P < 0.001$, Cox proportional hazards model).

Histopathologic evaluation of brain tissue was done in all animals at death or after study sacrifice. Animals showing clinical signs of tumor progression were euthanized. Of the 10 animals surviving to study end at day 100, which only occurred within the groups receiving nanoliposomal CPT-11 at 0.06, 0.4, 0.8, and 1.6 mg/

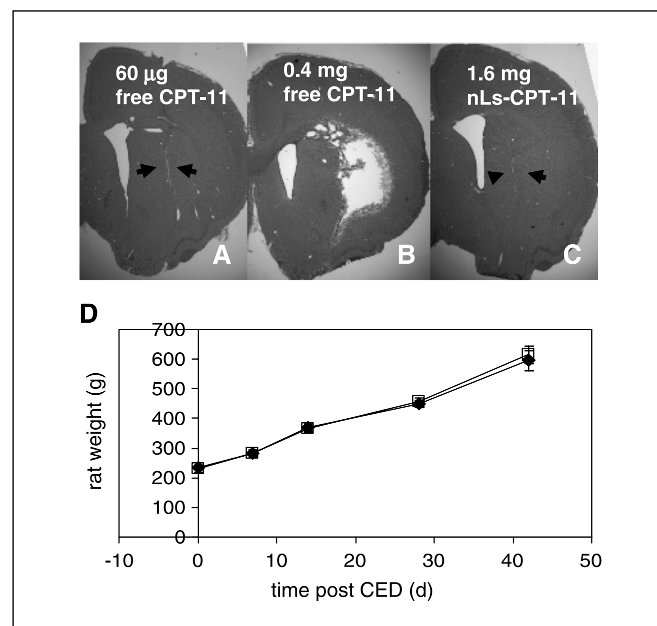

Figure 3. Tissue toxicity of free CPT-11 and nanoliposomal CPT-11 in the normal adult rat brain following CED. *A,* rats (four per group) treated with a single CED infusion of free CPT-11 at 60 μg (3 mg/mL) using an infusion volume of 20 μL. *B,* rats (four per group) treated with a single CED infusion of free CPT-11 at 0.4 mg (20 mg/mL) using an infusion volume of 20 μL. *C,* rats (four per group) treated with a single CED infusion of nanoliposomal CPT-11 at 1.6 mg (80 mg/mL) using an infusion volume of 20 μL. Six weeks after CED, animals were sacrificed and brains were processed for histopathology. Representative H&E sections from each group. Extensive tissue injury was observed in all animals treated with 0.4 mg free CPT-11. *Arrows,* rats in other treatment groups showed only focal traumatic injury at the site of the infusion cannula. *D,* serial weight measurements in control rats (□) and rats given 1.6 mg nanoliposomal CPT-11 by CED (♦). *Bars,* SD.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/5/2801/2559783/2801.pdf by guest on 05 February 2023

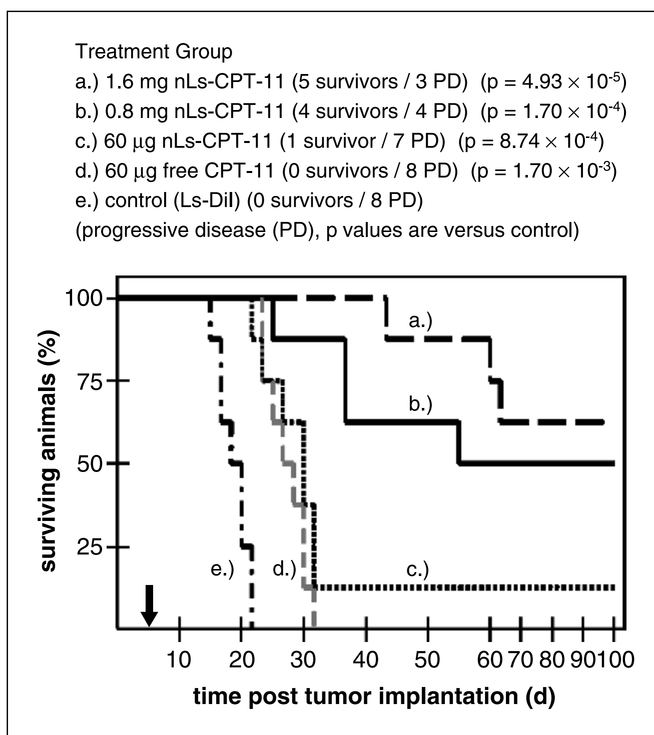

Figure 4. Treatment of rats bearing orthotopic U87 tumors with single CED infusion of free or nanoliposomal CPT-11. Five days after tumor implantation within the brain (*arrow*), rats were treated with nanoliposomal CPT-11 at 1.6 mg (80 mg/mL; *a*), nanoliposomal CPT-11 at 0.8 mg (40 mg/mL; *b*), nanoliposomal CPT-11 at 60 µg (3 mg/mL; *c*), free CPT-11 at 60 µg (3 mg/mL; *d*), and liposomal $DiIC_{18}(3)$ without encapsulated drug (empty liposomes; *e*). Eight animals per group. Median survival for each group was >100 days (*a*), 78 days (*b*), 30 days (*c*), 28.5 days (*d*), and 19.5 days (*e*).

rat, only 1 rat (0.8 mg/rat) showed histologic evidence of residual brain tumor; complete pathologic responses were noted in the 9 other survivors (Fig. 5*A* and *B*). In all cases of animal death, tumor progression was observed in the brains of the rats (Fig. 5*C* and *D*).

Discussion

We reported previously that liposomes in the range of 40 to 100 nm can be efficiently infused by CED into large volumes within the brains of rodents (23, 24) and primates (25, 26), indicating the feasibility of this approach as a drug delivery strategy. We now describe, to our knowledge, the first such example of a liposome- or nanoparticle-based drug given via CED for brain tumor treatment in preclinical models. These results indicated that combining the novel agent nanoliposomal CPT-11, which features highly efficient and stable encapsulation of CPT-11 in lipidic nanoparticles, with CED provided significant anticancer activity with a large therapeutic index against brain tumors.

These studies confirmed the difficulties associated with systemic chemotherapy for brain tumor treatment. I.v. administration of free CPT-11, a drug that seems promising against brain tumors (34), resulted in very low drug concentrations within the rat CNS due to rapid systemic clearance within hours as well as limited blood-brain barrier penetration. The problem of low therapeutic levels is compounded by the considerable systemic toxicities of this drug in its free form (35). CED is designed to provide high local-regional drug levels while reducing systemic exposure, and this was evidenced in these studies. In human brain tumors, disruption of the tumor-blood barrier could increase systemic access of this agent following CED; however, animals receiving CED of nanoliposomal CPT-11 at the highest dose tested (1.6 mg/rat, 80 mg/mL CPT-11/HCl) showed undetectable drug levels in plasma. Furthermore, this CED dose amounted to ~18-fold less total drug than an i.v. injection of free CPT-11 at 100 mg/kg (29.3 mg CPT-11/HCl). Finally, the slow and sustained release of drug from nanoliposomal CPT-11 provides an additional safety margin upon any systemic exposure.

Compared with systemic therapy, CED of free CPT-11 yielded much higher drug levels in brain tissue (17-fold) and even greater drug concentrations when CED was combined with nanoliposomal CPT-11 (341-fold). Furthermore, whereas CED of free CPT-11 was cleared from the brain quickly (<1 day), nanoliposomal CPT-11 was retained for many days in a dose-dependent manner; a single CED administration at 1.6 mg/rat was detectable over weeks (>49 days). Encapsulation of CPT-11 in these nanoliposome constructs resulted in at least 196-fold prolongation of residence time in normal tissue.

It is striking that greatly prolonged tissue retention of nanoliposomal CPT was associated with a substantial decrease in CNS toxicity compared with free drug and indeed was well tolerated up to the highest dose tested, 1.6 mg/rat. Since significant CNS toxicity was observed with free CPT-11 at 0.4 mg/rat, encapsulation in the nanoliposome construct increased tissue tolerance by

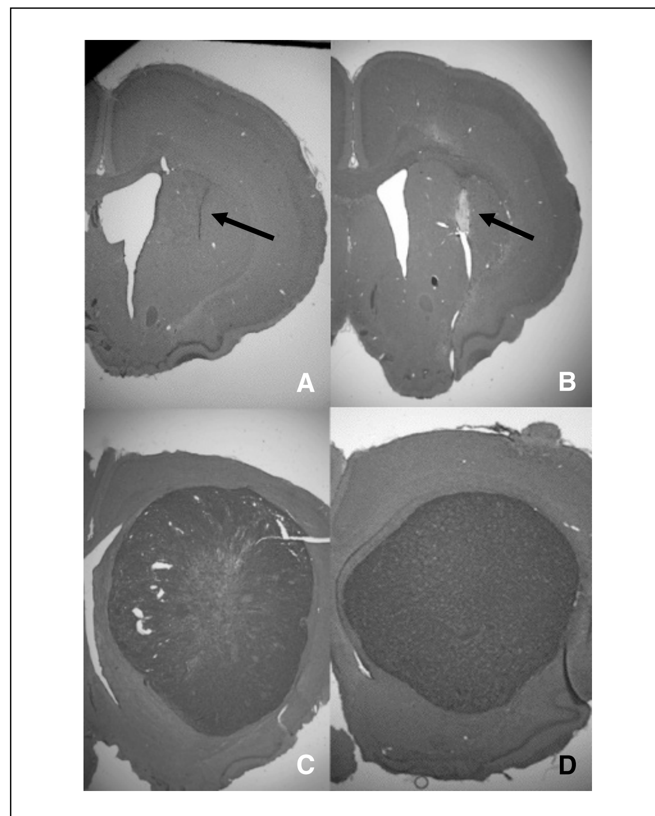

Figure 5. Representative brain sections from each experimental group in the therapy study. *A,* 1.6 mg nanoliposomal CPT-11 (obtained from a survivor and showing no residual tumor). *B,* 0.8 mg nanoliposomal CPT-11 (obtained from a survivor and show no residual tumor). *C,* 60 µg free CPT-11 (showing a typical tumor found in nonsurviving animals, in which tumor progression led to death). *D,* liposomal $DiIC_{18}(3)$ (empty liposomes; showing a typical tumor found in nonsurviving animals, in which tumor progression led to death). *Arrows,* scar from the cannula used for tumor implantation and CED.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/5/2801/2559783/2801.pdf by guest on 05 February 2023

>4-fold. This protective effect may be due to lower acute tissue exposure associated with particle encapsulation and its attendant slow rate of drug release.

Although equivalent CED doses of free CPT-11 and nanoliposomal CPT-11 indicated a slight but nonsignificant survival benefit for the latter, the treatment advantage provided by the nanoliposome construct was largely attributable to its much wider therapeutic index, enabling significantly higher doses with no added toxicity. It is possible that other mechanisms of delivery may also have contributed to the observed anticancer efficacy. Following initial distribution within brain tissue by CED, liposomes provided sustained drug levels for a prolonged period, resulting in continuous tumor exposure and a metronomic chemotherapy effect.

Nanoliposomal CPT-11 is a novel lipidic nanoparticle carrier that may be particularly well suited for brain tumor treatment. CPT-11 is very active against brain tumor cells (36, 37) and is being evaluated in clinical trials despite its pharmacologic limitations (19, 38). Using a sucrose octasulfate-based method for intraliposomal loading and stabilization, nanoliposomal CPT-11 provided clearly superior drug delivery and efficacy in conjunction with CED infusion. The highly concentrated dose of CPT-11 achieved (up to 1.6 mg/rat) was a direct result of the extremely high drug-to-lipid ratio ($\sim$800 g CPT-11/mol phospholipid, 1×10^5 CPT-11 molecules per liposome particle) attainable using this drug loading technology. This system can also, in principle, be used to deliver other drugs and/or diagnostic agents. For example, we have reported previously that similar liposomes loaded with gadolinium chelates can be readily visualized by real-time magnetic resonance imaging in the rodent (24) and monkey (25) CNS.

We conclude that nanoliposomal CPT-11 can be infused by CED into the CNS, resulting in substantial improvement in pharmacologic profile and therapeutic index. The strategy of combining a novel nanoparticle chemotherapeutic with CED may circumvent the drug delivery barriers posed by brain tumors.

Acknowledgments

Received 10/3/2005; revised 12/5/2005; accepted 12/12/2005.

Grant support: National Cancer Institute Specialized Programs of Research Excellence in Brain Tumors grant P50-CA097257 and Accelerated Brain Cancer Cure; New Investigator Award from California's Breast Cancer Research Program of the University of California grant 7KB-0066 (D.C. Drummond).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Dr. Dan Moore (University of California at San Francisco) for expert biostatistical assistance.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/5/2801/2559783/2801.pdf by guest on 05 February 2023

References

1. Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH. Convection-enhanced delivery of macromolecules in the brain. Proc Natl Acad Sci U S A 1994;91:2076–80.

2. Morrison PF, Chen MY, Chadwick RS, Lonser RR, Oldfield EH. Focal delivery during direct infusion to brain: role of flow rate, catheter diameter, and tissue mechanics. Am J Physiol Regul Integr Comp Physiol 1999;277:R1218–29.

3. Pardridge WM. Drug and gene targeting to the brain with molecular Trojan horses. Nat Rev Drug Discov 2002;1:131–9.

4. Pardridge WM. Blood-brain barrier drug targeting: the future of brain drug development. Mol Interv 2003;3:90–105.

5. Kunwar S. Convection-enhanced delivery of IL13-38QQR for treatment of recurrent malignant glioma: presentation of interim findings from ongoing phase 1 studies. Acta Neurochir Suppl 2003;88:105–11.

6. Lidar Z, Mardor Y, Jonas T, et al. Convection-enhanced delivery of paclitaxel for the treatment of recurrent malignant glioma: a phase I/II clinical study. J Neurosurg 2004;100:472–9.

7. Mardor Y, Roth Y, Lidar Z, et al. Monitoring response to convection-enhanced Taxol delivery in brain tumor patients using diffusion-weighted magnetic resonance imaging. Cancer Res 2001;61:4971–3.

8. Poepperl G, Goldbrunner R, Gildehaus FJ, Tanner P, Tatsch K. F-18 FET PET for monitoring the effects of convection-enhanced delivery of paclitaxel in patients with recurrent glioblastoma. Eur J Nucl Med Mol Imaging 2005;32:1018–25.

9. Gabizon A, Isacson R, Libson E, et al. Clinical studies of liposome-encapsulated doxorubicin. Acta Oncol 1994; 33:779–86.

10. Zucchetti M, Boiardi A, Silvani A, Parisi I, Piccolrovazzi S, D'Incalci M. Distribution of daunorubicin and daunorubicinol in human glioma tumors after administration of liposomal daunorubicin. Cancer Chemother Pharmacol 1999;44:173–6.

11. Thigpen JT, Aghajanian CA, Alberts DS, et al. Role of pegylated liposomal doxorubicin in ovarian cancer. Gynecol Oncol 2005;96:10–8.

12. Zamboni WC, Ramalingam S, Friedland DM, et al. Phase I and pharmacokinetic (PK) study of STEALTH® liposomal CKD-602 (S-CKD602) in patients with advanced solid tumors. American Society of Clinical Oncology Annual Meeting; 2005; Orlando, FL. p. 2069.

13. Seiden MV, Muggia F, Astrow A, et al. A phase II study of liposomal lurtotecan (OSI-211) in patients with topotecan resistant ovarian cancer. Gynecol Oncol 2004;93:229–32.

14. Gelmon KA, Tolcher A, Diab AR, et al. Phase I study of liposomal vincristine. J Clin Oncol 1999;17:697–705.

15. Drummond DC, Noble CO, Mamot C, et al. Development of novel liposomal and receptor tyrosine kinase-targeted immunoliposomal vinorelbine formulations with improved drug retention [abstract]. Proc Am Assoc Cancer Res 2005;56:4394.

16. Drummond DC, Noble CO, Guo Z, et al. Development of a highly active nanoliposomal irinotecan using a novel intraliposomal stabilization strategy. Cancer Res. In press 2006.

17. Drummond DC, Marx C, Guo Z, et al. Enhanced pharmacodynamic and antitumor properties of a histone deacetylase inhibitor encapsulated in liposomes or ErbB2-targeted immunoliposomes. Clin Cancer Res 2005;11:3392–401.

18. Gilbert MR, Supko JG, Batchelor T, et al. Phase I clinical and pharmacokinetic study of irinotecan in adults with recurrent malignant glioma. Clin Cancer Res 2003;9:2940–9.

19. Friedman HS, Petros WP, Friedman AH, et al. Irinotecan therapy in adults with recurrent or progressive malignant glioma. J Clin Oncol 1999;17:1516–25.

20. Chou R, Chen A, Lau D. Complete response of brain metastases to irinotecan-based chemotherapy. J Clin Neurosci 2005;12:242–5.

21. Mathijssen RHJ, van Alphen RJ, Verweij J, et al. Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). Clin Cancer Res 2001;7:2182–94.

22. Lokiec F, duSorbier BM, Sanderink GJ. Irinotecan (CPT-11) metabolites in human bile and urine. Clin Cancer Res 1996;2:1943–9.

23. Mamot C, Nguyen JB, Pourdehnad M, et al. Extensive distribution of liposomes in rodent brains and brain tumors following convection-enhanced delivery. J Neurooncol 2004;68:1–9.

24. Saito R, Bringas JR, McKnight TR, et al. Distribution of liposomes into brain and rat brain tumor models by convection-enhanced delivery monitored with magnetic resonance imaging. Cancer Res 2004;64:2572–9.

25. Saito R, Krauze MT, Bringas J, et al. Gadolinium-loaded liposomes allow for real-time magnetic resonance imaging of convection-enhanced delivery in the primate brain. Exp Neurol 2005;196:381–9.

26. Krauze MT, McKnight TR, Yamashita Y, et al. Real-time visualization and characterization of liposomal delivery into the monkey brain by magnetic resonance imaging. Brain Res Prot 2005;16:20–6.

27. Krauze MT, Saito R, Noble C, et al. Effects of the perivascular space on convection-enhanced delivery of liposomes in primate putamen. Exp Neurol 2005;196: 104–11.

28. Bartlett GR. Phosphorous assay in column chromatography. J Biol Chem 1959;234:466–8.

29. Bankiewicz KS, Eberling JL, Kohutnicka M, et al. Convection-enhanced delivery of AAV vector in parkinsonian monkeys; *in vivo* detection of gene expression and restoration of dopaminergic function using pro-drug approach. Exp Neurol 2000;164:2–14.

30. Cao SS, Rustum YM. Synergistic antitumor activity of irinotecan in combination with 5-fluorouracil in rats bearing advanced colorectal cancer: role of drug sequence and dose. Cancer Res 2000;60:3717–21.

31. Escoriaza J, Aldaz A, Castellanos C, Calvo E, Giraldez J. Simple and rapid determination of irinotecan and its metabolite SN-38 in plasma by high-performance liquid chromatography: application to clinical pharmacokinetic studies. J Chromatogr B 2000;740:159–68.

32. Warner DL, Burke TG. Simple and versatile high-performance liquid chromatographic method for the simultaneous quantitation of the lactone and carboxylate forms of camptothecin anticancer drugs. J Chromatogr B 1997;691:161–71.

33. Zufia L, Aldaz A, Giraldez J. Separation methods for camptothecin and related compounds. J Chromatogr B 2001;764:141–59.

34. Friedman HS, Keir ST, Houghton PJ. The emerging role of irinotecan (CPT-11) in the treatment of malignant glioma in grain tumors. Cancer 2003;97:2359–62.

35. Rothenberg ML, Meropol NJ, Poplin EA, Van Cutsem E, Wadler S. Mortality associated with irinotecan plus bolus fluorouracil/leucovorin: summary findings of an independent panel. J Clin Oncol 2001;19:3801–7.

36. Chen TC, Su S, Fry D, Liebes L. Combination therapy with irinotecan and protein kinase C inhibitors in malignant glioma. Cancer 2003;97:2363–73.

37. Nakatsu S, Kondo S, Kondo Y, et al. Induction of apoptosis in multi-drug resistant (MDR) human glioblastoma cells by SN-38, a metabolite of the camptothecin derivative CPT-11. Cancer Chemother Pharmacol 1997;39:417–23.

38. Reardon DA, Friedman HS, Powell JBJ, Gilbert M, Yung WK. Irinotecan: promising activity in the treatment of malignant glioma. Oncology 2003;17:9–14.