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Laboratory Investigation

Polymer delivery of camptothecin against 9L gliosarcoma: release, distribution, and efficacy

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Summary

Camptothecin is a potent antineoplastic agent that has shown efficacy against multiple tumor lines *in vitro*; unfortunately, systemic toxicity has limited its *in vivo* efficacy. This is the first study to investigate the release, biodistribution, and efficacy of camptothecin from a biodegradable polyanhydride polymer. Tritiated camptothecin was incorporated into biodegradable polymers that were implanted intracranially in 16 male Fischer 344 rats and the animals were followed up to 21 days post-implant. A concentration of 11–45 μg of camptothecin-sodium/mg brain tissue was within a 3 mm radius of the polymer disc, with levels of 0.1 μg at the outermost margin of the rat brain, 7 mm from the site of implantation. These tissue concentrations are within the therapeutic ranges for human and rat glioma lines tested against camptothecin-sodium *in vitro*. The *in vivo* efficacy of camptothecin-sodium was evaluated with male Fischer 344 rats implanted intracranially with 9L gliosarcoma and compared with the efficacy of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). The animals were divided into four groups. Group 1 (control) had a median survival of 17 days. Group 2 (3.8% BCNU polymer) had a median survival of 23 days ($P = 0.006$). Group 3 (20% camptothecin polymer) had a median survival of 25 days ($P = 0.023$). Group 4 (50% camptothecin polymer) had a median survival of 69 days ($P < 0.001$). Drug loadings of 20% and 50% camptothecin released intact camptothecin for up to 1000 h *in vitro*. We conclude that the biodegradable polymer p(CPP : SA) releases camptothecin-sodium, produces tumoricidal tissue levels, results in little or no systemic toxicity, and prolongs survival in a rat glioma model.

Introduction

Camptothecin is a plant alkaloid isolated from stem wood of the Chinese tree, *Camptotheca acuminata* [1]. It is a cytotoxic alkaloid with strong antitumor activity against a wide variety of experimental tumors [1–4]. The intracellular target is eukaryotic DNA topoisomerase I [5–7], an enzyme that catalyzes the relaxation of supercoiled DNA by creating transient single strand DNA breaks, which are subsequently ligated by the enzyme [8]. Camptothecin stabilizes the complex between topoisomerase I and DNA, thus allowing enzyme-linked DNA breaks but preventing ligation [5,9]. Knab et al. [10] further supported the theory that topoisomerase I is the target for camptothecin by mutating topoisomerase I, which created camptothecin resistant cells. For cytotoxicity camptothecin must exist as a closed-ring lactone; when the lactone ring is opened by hydrolysis, the drug is inactivated [11–13].

Because of the potent antitumor activity of camptothecin, clinical trials were initiated in the late 1960s [14–17] with camptothecin-sodium, a water-soluble form of the drug. Even though the camptothecin-sodium is in the open-ring inactive form, it remains cytotoxic because it is in equilibrium with the active form at physiologic pH [18,19]. These clinical trials showed antitumor effect, but significant toxicity to bone marrow and nonhematologic systems caused the trials to be aborted.

To maximize antitumor effect and minimize systemic toxicity, we incorporated camptothecin-sodium into a food and drug administration (FDA)-approved, implantable, biodegradable polyanhydride

polymer matrix poly[bis(*p*-carboxypenoxy)propane-sebacic acid] copolymer p(CPP : SA)(20 : 80) that locally delivers the drug to the tumor bed (from this point forward we will refer to camptothecin-sodium as camptothecin).

Methods and materials

Polymer preparation

p(CPP : SA)(20 : 80) polymers containing camptothecin and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) were synthesized according to the method of Domb and Langer [20]. The National Cancer Institute (Bethesda, MD) provided the camptothecin. Combining camptothecin with p(CPP : SA)(20 : 80) produced a 20% or 50% camptothecin polymer mixture by weight. Adding methylene chloride dissolved the mixture and yielded a 10% solution (w/v). Desiccating the solution in a vacuum for 72 h yielded a dry powder. Camptothecin polymer discs (10 mg final weight) were prepared by compression molding 10.5–11.0 mg of the camptothecin polymer powder with a stainless steel mold (internal diameter, 2.5 mm) under light pressure from a Carver Press at 200 psi as described by Leong et al. [21,22]. 3.8% loaded BCNU p(CPP : SA)(20 : 80) discs of the same size were synthesized by co-dissolving BCNU and polymer in methylene chloride, and then drying, and formulating into discs by compression molding as described above.

Release kinetics

Measuring the release of camptothecin involved placing camptothecin loaded polymers into a scintillation vial with 1.0 ml of 0.1 M phosphate-buffered saline, pH 7.4, and incubating at 37°C. The solution was removed at various time points and replaced with fresh buffer. High-pressure liquid chromatography (HPLC) measured the amount of camptothecin released into the buffer, as previously described [23]. Briefly, the Beckman chromatographic system had a 507 autosampler, 126 AA solvent module, 166 Detector, a reverse phase microBondpak C18 Waters column (Milford, MA, particle size 10 μm), and a System Gold data system. The HPLC system was eluted isocratically with methanol : water (63 : 37; v/v) at room temperature at a flow rate of 1.0 ml/min and measured the samples at a wavelength of 370 nm. Constructing a standard curve required plotting the peak area against concentration.

Animals

Male Fischer 344 rats weighing 200–250 g were obtained from Harlan Sprague–Dawley, INC. (Indianapolis, IN), kept in standard animal facilities, and maintained on Purina Certified Rodent Chow with free access to Baltimore City water.

Anesthesia

An i.p. injection of 2–4 ml/kg of a stock solution containing ketamine hydrochloride (25 mg/ml), xylazine (2.5 mg/ml), and 14.25% ethyl alcohol in normal saline anesthetized the rats. For euthanasia, rats received an intracardiac injection of 0.3 ml of Euthanasia-6 Solution CII (Veterinary Laboratories, Inc., Lenexa, KS).

Intracerebral drug distribution

Polymer discs were prepared as described above, except that ^{3}H-labeled camptothecin-sodium (specific activity, 73.5 mCi/mmol; Research Triangle Institute) was added to the initial solution of polymer and camptothecin in methylene chloride. Twelve rats received implants of 10-mg discs containing 50% camptothecin by weight and 1.5 μCi/mg disc weight.

The procedure for polymer implantation in the rat is described elsewhere [24]. Briefly, anesthetized rats had their heads shaved and prepared aseptically. Exposing the dura required a midline incision and a 3-mm burr hole drilled through the skull 5 mm posterior and 3 mm lateral to the bregma. Incising the dura with a microsurgical knife exposed the brain parenchyma and allowed the insertion of a polymer disc. The wound was irrigated and closed with surgical clips (Cay Adams, Parsippany, NJ).

At 1, 3, 7, and 21 days after implantation, groups of three rats each were euthanized. The polymer disc was removed so that the brain could then be snap frozen in hexane over dry ice, and sectioned in the midline into implant and contralateral hemispheres. Each hemisphere was sectioned in the coronal plane at 2-mm intervals by using a tissue blade grid consisting of individual tissue blades arranged in parallel separated by 2-mm metal spacers. Each section was weighed, dissolved in 1.5 ml of Solvable homogenizing solution (New England Nuclear Dupont, Boston, MA), and combined with 15 ml of Atomlight scintillation mixture (Packard). A Beckman liquid scintillation counter calculated the dpm of the brain samples.

A second experiment allowed conversion of dpm/mg tissue to camptothecin concentration. Four rats received implants of 50% loaded polymer discs with 1.5 μCi/mg. One rat each was killed at 1, 3, 7, and 21 days. After removing and freezing the brain as described above, we cut 2-mm coronal sections through the site of the polymer site. The section was minced and extracted with ethanol. We divided the ethanol fraction in two, dried them in a vacuum desiccator and then resuspended them in 100 ml of ethanol. Samples of this solution, as well as a solution of nonradiolabeled camptothecin, were spotted on silica thin layer chromatography plates. We developed the plate with methylene chloride : methanol (95 : 5) and exposed it in an iodine chamber. Calculating the R_f value for camptothecin involved cutting each lane into four sections: A, origin; B, origin to camptothecin spot; C, camptothecin; and D, camptothecin spot to solvent front. A liquid scintillation counter determined counts of the chromatography strips. The distribution of labeled camptothecin across the chromatography plate allowed determination of signal corresponding to intact drug. To determine the efficiency of extraction, the remaining half of the original extract was combined with Atomlight mixture and counted, and the residual brain tissue was homogenized and counted as above. Calculating the concentration of camptothecin in ng/mg brain tissue required multiplying the percentage of intact camptothecin by the dpm/mg of brain and dividing by the specific activity of camptothecin present in the polymer disc.

Intracranial model

The 9L gliosarcoma was maintained in the flanks of male Fischer 344 rats. The tumor was initially obtained in 1985 from the Brain Tumor Research Center, University of California at San Francisco, CA and was passed every 2–3 weeks. For intracranial implantation, the tumor was surgically excised from the carrier animal after the animal was antiseptically prepared with 70% ethanol and Prepodyne solution. The tumor was then cut into 1-mm^3 pieces that were placed in sterile 0.9% NaCl and kept on ice during the implantation procedure.

Implant toxicity

To study the toxicity of the polymer camptothecin implant, we implanted intracranially in rats p(CPP : SA) discs containing 20% and 50% camptothecin by weight, as described above. Control rats received blank p(CPP : SA) discs without camptothecin. The rats were examined twice daily for signs of neurotoxicity with respect to grooming and gait. After 200 days, all surviving rats were killed and their brains were removed and fixed in formalin. One coronal brain section centered through the polymer implant was taken for each rat and stained with hematoxylin and eosin. A blinded neuropathologist examined the histology.

Efficacy study

We tested the efficacy of the camptothecin polymer implant against the 9L gliosarcoma tumor model and compared it against the efficacy of a BCNU polymer implant in the same experiment. Forty Fischer 344 rats were anesthetized with an i.p. injection, and the surgical site was prepared by shaving and applying 70% EtOH and Prepodyne solution. Investigators made a midline incision on the posterior aspect of the animal's head and identified the coronal suture. Five millimeters posterior to the coronal suture and 3 mm lateral to the sagittal suture on the left parietal bone investigators made a 3-mm burr hole. The dura was opened with a cruciate incision and the underlying cortex and white matter were gently aspirated until the superior aspect of the brainstem was exposed. Packing the wound with gauze and a silver nitrate applicator controlled bleeding. After achieving hemostasis, a 1-mm^3 9L gliosarcoma tumor piece was placed into the defect. The wound was irrigated and closed with surgical clips. Five days after tumor implantation the rats were randomized into one of four groups, one control group and three treatment groups. After reanesthetizing the rats the wounds were reopened under sterile conditions. After confirming tumor presence, each animal underwent implantation of a 10 mg p(CPP : SA)(20 : 80) disc containing no drug (control group), 20% camptothecin, 50% camptothecin or 3.8% BCNU (a concentration shown to prolong survival in phase III clinical trials) [25]. After achieving hemostasis, the wounds were closed with surgical clips.

For direct injection, camptothecin was dissolved in sterile 0.9% NaCl to yield a 100 mg/ml solution. Five days after implantation, the 20 rats were randomized into two groups of 10, one control and one treatment. The animals were placed into a stereotactic frame and the treatment group received 50 μl of the camptothecin solution injected at a depth of 3.5 mm from the surface. Control animals received 50 μl of 0.9% saline. After

injection, the burr hole was covered with bone wax to prevent efflux.

Investigators evaluated the rats on a daily basis for signs of neurological changes. Animals showing signs of neurological compromise, or an inability to feed or groom themselves were euthanized. At the time of death investigators removed the animals' brains and placed them in 10% formalin for at least 7 days. Coronal sections were taken through the polymer implant site and stained with hematoxylin and eosin to evaluate for the presence of tumor.

Animals surviving to the end of the study (120 days) were 'long-term' survivors.

Statistical analysis

Survival was plotted on a Kaplan–Meier survival curve and statistical significance was determined by a nonparametric Kruskal–Wallis analysis of variance followed by a nonparametric Wilcoxon Rank Sum Test [26]. Values were considered significant for $P < 0.05$.

Results

In vitro *drug release*

Release of camptothecin from biodegradable p(CPP : SA)(20 : 80) polymers is shown as a cumulative percentage of the total drug loaded into the polymer in Figure 1. For each of the two camptothecin loadings (20% and 50%), a large initial release of camptothecin occurred over the first 36–48 h. The initial, rapid release was followed by a steady but considerably slower rate of release over the next 25 days. At the end of the experiment, the 20% loaded polymer released 9% of its loading dose (0.18 mg of camptothecin) into the buffer, while the 50% loaded polymer released 40% of its loading dose (2.0 mg of camptothecin). The initial release of the 50% loaded polymer accounted for the order of magnitude increase in total camptothecin release compared to the 20% loaded polymer. After the initial release, both polymers approximate zero-order kinetics for the remainder of the experiment. Because the *in vivo* release of camptothecin from a biodegradable polymer into the brain may be considerably different from the *in vitro* release into a buffer, we studied the intrathecal distribution of tritiated camptothecin.

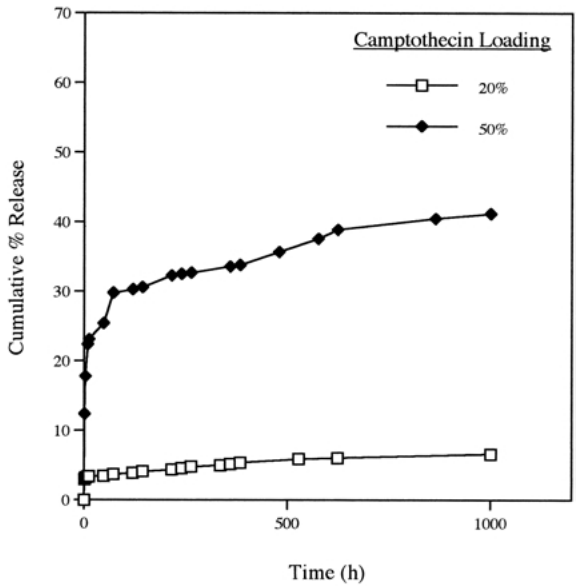

Figure 1. In vitro release kinetics of camptothecin released from p(CPP : SA)(20 : 80) polymer discs (10 mg) loaded with 20% and 50% camptothecin by weight. Each point represents the mean of three measurements.

Intracerebral distribution

We evaluated the intrathecal distribution of a 50% loaded camptothecin polymer because this dose was nontoxic and the most effective camptothecin dose (see below). Figure 2 shows the distribution of camptothecin throughout the rat brain after release from the p(CPP : SA)(20 : 80). All sections of the brain had detectable amounts of camptothecin as early as day 1, with the greatest concentration within 3 mm of the polymer implant on the ipsilateral hemisphere. At day 21, concentrations ranged from 11 to 49 μg camptothecin/mg brain. A drop occurred in the concentration of camptothecin in the brain sections more than 3 mm away from the implant. A concentration of 100 ng/mg brain was detected at the edge of the brain, 7 mm from the implant, showing measurable concentrations of drug throughout the entire brain. Even though a concentration of 100 ng/mg brain may not be cytotoxic, *in vitro* studies show that cytotoxicity of camptothecin against gliomas increases as the exposure is prolonged [23]. The concentrations of camptothecin in the contralateral hemisphere were more uniformly distributed than those in the ipsilateral hemisphere and similar to the concentrations in the ipsilateral hemisphere at a distance of 5 mm or greater from

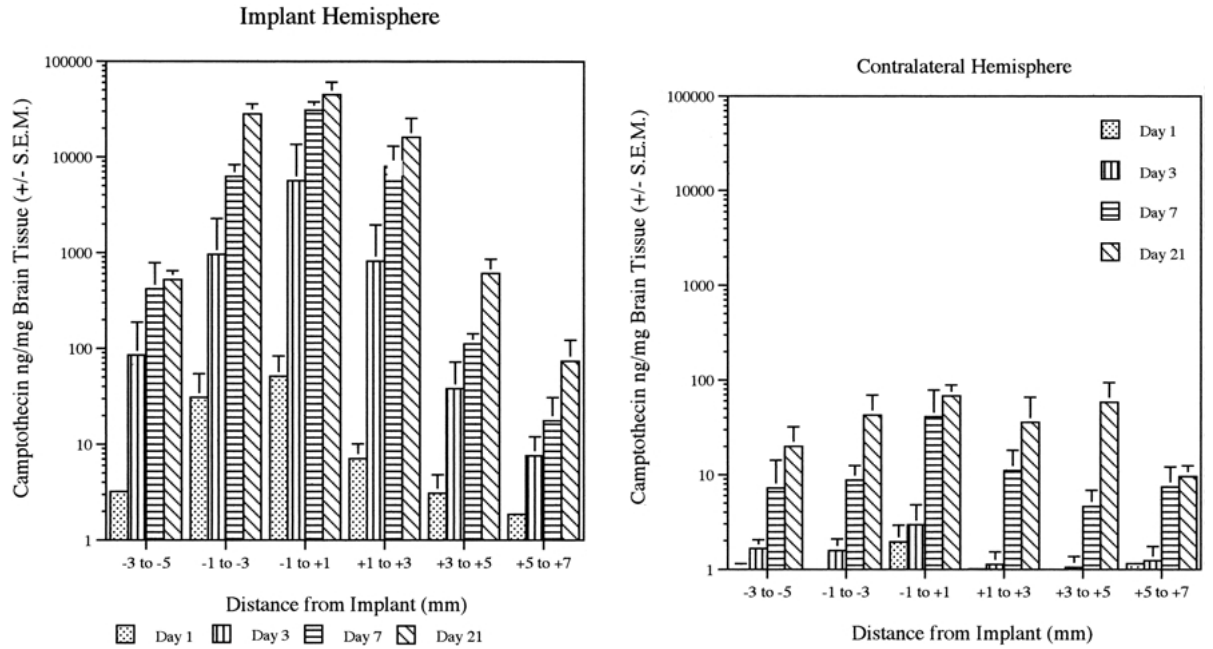

Figure 2. Ipsilateral and contralateral intracerebral distribution of tritiated camptothecin in the rat brain released from p(CPP : SA) polymer loaded with 50% camptothecin by weight. The distance from the implant is in the coronal plane with the positive distances anterior to the implant and the negative distances posterior to the implant. Sections were carried out to the edge of the brain in both directions. Each distance represents the mean of three animals.

the implant. The highest percentage of drug release occurred within the first week, but the camptothecin concentrations increased in all sections of the brains throughout the 21 days. We determined the percentage of radioactivity corresponding to camptothecin in each slice using thin layer chromatography. Approximately, 46 ± 6% (standard error of the mean) of the raw counts represented free camptothecin. The remaining raw counts were from metabolites (26 ± 6%), and tissue-bound drug (28 ± 5%).

Implant toxicity

There were no clinical manifestations of toxicity from the camptothecin polymer implants. We randomized the animals into three groups and they received a blank polymer, or 20% or 50% loaded camptothecin polymer. Unblinded observers assessed all of the animals twice daily for motor activity, gait, grooming, and weight loss. The animals implanted with camptothecin polymers were indistinguishable from controls and remained neurologically intact throughout the experiment. On day 200, the animals were killed and their brains were cut in 2-mm coronal sections and representative samples from each section were stained with hematoxylin and eosin. A neuropathologist, who was blinded to the therapy, performed histological examination of brain tissue sections. The reviewer consistently distinguished the samples treated with camptothecin polymers from animals treated with blank polymers. The samples through the camptothecin polymer implant showed an area of coagulation necrosis and dystrophic calcification, as well as hemosiderin surrounding thickened blood vessels. Samples from the adjacent sections also showed coagulation necrosis, but sections greater than 3 mm from the implant did not demonstrate coagulation necrosis. These histologic changes were not seen in animals receiving blank p(CPP : SA)(20 : 80) polymers. The reviewer, however, was unable to distinguish animals that were treated with 20% camptothecin polymers from animals that were treated with 50% camptothecin polymers due to a lack of quantitative or qualitative differences in the histological findings. This suggests that the histologic findings are a result of the camptothecin exposure, but that the greater concentration of drug release from the 50% camptothecin polymers is not reflected in greater injury to the brain based on histology. Further, the absence of morbidity and mortality suggests that the cytopathological changes are not manifested clinically.

Intracranial efficacy

We evaluated the efficacy of the camptothecin polymer in a 9L gliosarcoma model. Our 9L model is highly reproducible, and the survival curves from preclinical testing of polymer delivery of BCNU in the 9L model [27] closely paralleled the survival curves of polymer delivery of BCNU in phase III clinical trials [28]. We studied the efficacy of the camptothecin polymer implant against not only an empty polymer group (control) but also a group treated with 3.8% BCNU polymers (3.8% BCNU polymers have significantly extended survival in phase III clinical trials for patients with both newly diagnosed gliomas [28] and recurrent gliomas [25]). Because of an absence of clinical toxicity in the 50% camptothecin polymer group, we hypothesized that this group would be the most effective. We based this on the fact that the 50% camptothecin polymer released a greater mass of drug *in vitro* than the 20% camptothecin polymer. Because we appreciate that *in vivo* release kinetics can be much different than *in vitro* release profiles, we also wanted to compare a lower loading dose of camptothecin (we chose 20%) to the 50% camptothecin polymer to determine if the difference in their *in vitro* release kinetics translated into a difference in survival. Each group had 10 animals. The Kaplan–Meier survival curves are shown in Figure 3. The 50% camptothecin polymer significantly extended survival compared to the control group ($P < 0.001$) and the 3.8% BCNU group ($P < 0.001$), and resulted in four long-term survivors. The 20% camptothecin polymer significantly extended survival compared to the control group ($P = 0.023$) but not compared to the 3.8% BCNU group ($P > 0.2$), although the 20% camptothecin polymer preparation resulted in one long-term survivor. The 3.8% BCNU group significantly extended survival compared to the control group ($P = 0.006$). The median survivals for the 50% and 20% camptothecin groups, and the 3.8% BCNU group were 69, 25, and 23 days, respectively. The median survival for the control group was 17 days.

In a separate experiment, 5 mg of camptothecin (the mass of camptothecin in the 50% loaded polymer) was placed directly into the 9L tumor mass instead of being incorporated into a polymer matrix. There was no difference in survival between this group and controls ($P > 0.2$). The median survival for the camptothecin group without a polymer matrix was 20 days and for the controls was 19 days (Table 1).

At the time of death, investigators inspected each animal brain for gross tumor and prepared the brains for microscopic H and E evaluation. The long-term survivors were killed on day 120. All of the animals that died spontaneously had both macroscopic and microscopic evidence of tumor. The long-term survivors had neither macroscopic nor microscopic evidence of tumor cells, but there were areas of coagulation necrosis and dystrophic calcification.

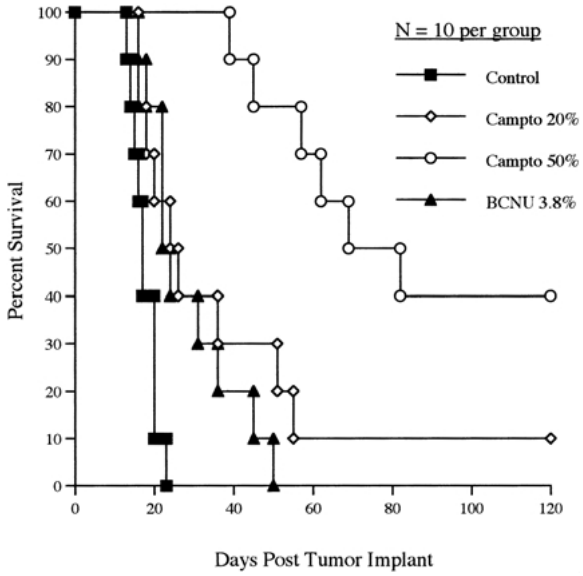

Figure 3. Kaplan–Meier survival curve. Fischer 344 rats received intracranial 9L gliosarcoma on day 0 and on day 5 they received an intratumor implant of a 10 mg p(CPP : SA) disc. The control animals received a blank disc while the other animals received 20% camptothecin, 50% camptothecin, or 3.8% BCNU. There are 10 animals per group.

Discussion

Delivering chemotherapeutic agents intracranially bypasses the blood–brain barrier and allows the drug to be delivered directly to the tumor bed. The blood–brain barrier and severe systemic toxicity have restricted potentially effective treatments of malignant gliomas with systemic chemotherapy. A myriad of chemotherapeutic agents have shown promising *in vitro* results against malignant glioma only to fail clinical trials because they cannot cross the blood–brain barrier in cytotoxic concentrations or because of unacceptable systemic toxicity.

Camptothecin has been previously demonstrated to have significant antineoplastic actions *in vitro* [29–31], but its clinical use as a systemic agent was limited by severe toxicity [14–17]. For this reason, we chose to

Table 1. Survival of rats treated with polymer delivery of camptothecin of BCNU

Experiment	Treatment	Number of rats	Median survival (days)	Number of long-term survivors	*P* value[a]	*P* value[b]
1	Control	10	17	0		
	CPT 20%	10	24	1	0.023	>0.2
	CPT 50%	10	69	4	<0.001	<0.001
	BCNU 3.8%	10	22	0	0.006	
2	Control	5	19	0		
	CPT 5 mg[c]	5	20	0	>0.2	

[a]Results of nonparametric Wilcoxon Rank Sum Test comparing treatment groups to controls.
[b]Results of nonparametric Wilcoxon Rank Sum Test comparing comptothecin groups to the 3.8% BCNU group.
[c]This group received 5 mg of camptothecin directly into the tumor without incorporation into a p(CPP : SA) polymer matrix (5 mg of camptothecin is the amount in the 50% loaded polymer).

study camptothecin in our local chemotherapy delivery model. Other factors making camptothecin an attractive agent include its unique mechanism of action (allowing for potential synergy and multiple drug therapy) as well as the availability of several camptothecin analogues, which appear to be even more potent antineoplastic agents (unpublished data).

In this study, we investigated the release, distribution, and efficacy of camptothecin delivered intracranially from a biodegradable polymer. The polymer is a polyanhydride copolymer matrix that has been tested for biocompatibility [22,32,33], and has been used to deliver the alkylating agent BCNU intracranially to patients with malignant gliomas and significantly extend their survival [23,25,34].

Polymer degradation proceeds with zero-order kinetics [34,35]; therefore, it was predicted that the amount of drug release would be constant with respect to time. Instead it was found that the *in vitro* drug release from the polymer matrix occurs with an initial, rapid-release phase followed by a slower and more constant release [36–38]. The *in vitro* data presented here also show this biphasic pattern. The initial, rapid-release phase is likely related to the presence of camptothecin on the surface of the polymer, particularly for the 50% loaded polymer, which contains equal amounts by weight of camptothecin and polymer. The 20% polymer had a less dramatic rapid-release phase, which would be expected because less drug would be on the surface of the polymer. After 72 h, the 50% loaded polymer released an order of magnitude more drug than the 20% loaded polymer. After the initial burst, the difference in the amount of drug released between the 50% and 20% loaded polymers was less pronounced. Both polymers continued to release drug throughout the 1000 h experiment.

Implanting the p(CPP : SA) polymer discs intracranially with tritiated camptothecin showed that camptothecin was distributed throughout the ipsilateral and contralateral rat brain. The distribution of tritiated camptothecin is explained by the work of Fung et al. [39]. They elucidated the pharmacokinetics of interstitial delivery of BCNU, 4-hydroperoxycyclophosphamide, and paclitaxel from a biodegradable polymer. They showed that edema, enhanced interstitial fluid convection (which is caused by an elevated pressure gradient between the brain interstitium and the ventricular space), and permeation through the blood–brain barrier (which may be leaky at the implant site and allow drug to enter the cerebral vasculature and then reenter the brain parenchyma), all contribute to a greater early distribution of drug than would be predicted by diffusion.

Camptothecin was detected 5–7 mm from the polymer even on day 1. Throughout the 3 weeks of the distribution study the concentration of camptothecin continued to increase, with the highest levels of camptothecin in the vicinity of the polymer implant. The continued release and accumulation of camptothecin over a 3-week period has important implications on efficacy. *In vitro* data shows that the LD_{90} concentration of camptothecin against human and rat glioma lines decreased by an order of magnitude with prolonged exposure [23]. This continued release maximizes the antitumor effect of camptothecin and explains why delivering camptothecin powder alone, directly to the tumor without a polymer matrix does not extend survival. Without the polymer matrix the drug can be more quickly removed from the tumor bed and the benefit of continued release of camptothecin is lost.

Despite the prolonged period of exposure to camptothecin there was no evidence of neurotoxicity and

there were only minimal histological changes in the brain. The efficacy and the lack of toxicity of camptothecin may be due to the presence of elevated topoisomerase I levels in the 9L gliosarcoma compared to levels in neurons and glia. This conclusion is supported by previous work using reverse transcriptase-polymerase chain reaction (RT-PCR) that showed a strong positive correlation between topoisomerase I levels and *in vitro* cytotoxicity of camptothecin [40,41]. Further, we found that rat and human glioma cell lines were two orders of magnitude more sensitive to camptothecin than astrocytes *in vitro* [40]. The increased resistance of astrocytes to camptothecin helps explain the lack of a histologic difference between the 50% and the 20% camptothecin polymers, even though the 50% polymers significantly extended survival compared to the 20% polymers. Both polymer doses released sufficient amounts of drug to cause cytotoxic changes at the polymer–brain interface, which is where the highest concentrations are reached (see Figure 2). The higher mass of drug released from the 50% polymer (see Figure 1) could be distributed at a concentration that is cytotoxic to glioma cells but not to astrocytes. The finding of overexpression of topoisomerase I is not limited to gliomas, it is also found in human colon adenocarcinomas and other malignancies, but not in normal tissues [41–43].

Our results indicate that the 50% ($P < 0.001$) and 20% ($P = 0.023$) camptothecin, and the 3.8% BCNU ($P = 0.006$) polymer implants significantly prolonged survival in a rat glioma model. Further, the 50% camptothecin group significantly extended survival compared to both the 20% camptothecin and the 3.8% BCNU groups ($P < 0.001$). We wanted to determine if the significant difference in survival between the 50% and 20% camptothecin groups could be explained by the difference in their *in vitro* release profiles. For example, could we further extend survival by delivering higher concentrations of camptothecin in the initial 24–72 h? Therefore, we placed camptothecin without a polymer matrix into rats containing 9L tumor. The mass of camptothecin (5 mg) was equivalent to the mass in the 50% loaded polymers. The survival curve of the treatment group was no different from the control group ($P > 0.2$). Even though the pharmacokinetics of the *in vivo* release are undoubtedly more complex, achieving, *in vitro*, an initial burst of camptothecin followed by sustained release seems to play an important role in its efficacy.

The safe and effective local delivery of camptothecin from biodegradable polymers avoids the unacceptable toxicity associated with systemic delivery and provides an important option as topoisomerase inhibitors are considered for the clinical armamentarium against malignant gliomas.

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