Polilactofate Microspheres for Paclitaxel Delivery to Central Nervous System Malignancies[1]

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ABSTRACT

Purpose: **The purpose of this study was to demonstrate that surgically implanted, controlled-release, biodegradable polilactofate microspheres (Paclimer) can be used safely to bypass the blood-brain barrier and deliver paclitaxel to malignant brain tumors.**

Experimental Design: **The rate of paclitaxel release from Paclimer microspheres submerged in PBS was measured *in vitro* by high-performance liquid chromatography. *In vivo* studies of Paclimer were performed as intracranial implants in Fischer 344 rats in the presence or absence of 9L gliosarcoma. Mantel-Cox statistics were used to assess the efficacy of Paclimer at extending survival of tumor-bearing animals compared with control implants. Paclimer implants tagged with [^{3}H]paclitaxel were used to measure biodistribution of paclitaxel from the Paclimer implant.**

Results: **Paclimer released paclitaxel at a constant rate for up to 3 months *in vitro*. *In vivo*, Paclimer implants placed intracranially in rats released active drug for up to 30 days after implantation and doubled the median survival of rats bearing established 9L gliosarcomas (median survival of paclitaxel-treated animals = 35 days; median survival of control-treated animal = 16 days; $P < 0.0001$). Active drug was distributed throughout the rat brain based on liquid scintillation counting and TLC. Rats implanted with Paclimer demonstrated no overt signs of neurotoxicity and exhibited local cytopathological changes consistent with exposure to an antimicrotubule agent.**

Conclusions: **Paclimer extends survival in a rodent model of glioma with minimal morbidity and optimal pharmacokinetics.**

INTRODUCTION

The blood-brain barrier is a major obstacle to developing effective pharmacological treatments for CNS[4] malignancies. Tight junctions unique to cerebral endothelial cells exclude most pharmaceutical agents that are not able to diffuse across their lipid bilayer. Recently, biodegradable, controlled-release, polymeric formulations have been developed that can be surgically implanted into a tumor, bypassing the blood-brain barrier (1). One of the formulations, PCPP-SA impregnated with the nitrosourea carmustine, has been used successfully for treatment of malignant brain tumors and became the first United States Food and Drug Administration-approved therapy for this disease in 23 years (2). In this report we describe another biodegradable, controlled-release, polymeric microsphere construct that is biocompatible in the CNS and extends the types of compounds that can be delivered intracranially *in vivo*. Polilactofate microspheres consist of a polyphosphoester polymer backbone that undergoes hydrolytic degradation in an aqueous environment, releasing drug encapsulated within the microspheres. Because the microsphere formulation of the polilactofate polymer provides a large surface area for hydrophobic degradation of the polymer, polilactofate microspheres are particularly suitable for delivery of high molecular weight, highly hydrophobic compounds that are released slowly or minimally from existing controlled-release matrices such as PCPP-SA. Paclitaxel, an antimicrotubule, antineoplastic agent, was evaluated in conjunction with the polilactofate matrix based on these physical properties (Paclimer Delivery System). Paclitaxel has been clinically effective against a variety of human cancers, including ovarian and breast cancer, and has been used in conjunction with polilactofate microspheres in animal models of non-small cell lung cancer (3). Unfortunately, paclitaxel has failed multiple clinical trials against malignant brain tumors [glioma (4–10)] because even at maximally tolerated systemic doses, levels in the CNS are low to undetectable (4, 11). Nevertheless, *in vitro* assays

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[4] The abbreviations used are: CNS, central nervous system; PCPP-SA, poly(carboxyphenoxypropane-sebacic acid).

demonstrate that brain tumor cell lines are sensitive to paclitaxel, indicating that clinical responses may be possible if adequate CNS paclitaxel concentrations can be achieved (12–14). Therefore, we undertook the present study to determine (*a*) whether polilactofate microspheres were biocompatible in the CNS and (*b*) whether they could deliver therapeutic levels of paclitaxel to CNS malignancies.

MATERIALS AND METHODS

Paclitaxel:Polymer Formulation. Paclitaxel was incorporated into the biodegradable polyphosphoester polymer p(DAPG-EOP) in the form of microspheres at 10% (w/w) loading by dissolving both substances in ethyl acetate and pumping the solution through an in-line homogenizer with 0.5% polyvinyl acetate solution to a container with an overhead stirrer. After the microspheres had hardened, they were filtered and lyophilized to give the Paclimer product. The size of the resultant Paclimer microspheres was determined by a single particle optical sensing system (Model 770; Particle Sizing Systems, Langhome, PA). The median diameter of the microspheres was 53 μm, with a range of 20–200 μm (3). The Paclimer microspheres were then combined with PEG-1000 at the desired ratio by weight in a 37°C water bath, mixed to obtain a uniform slurry, and cooled to 4°C. Ten-mg aliquots of the Paclimer/PEG-1000 suspension were pressed into discs for surgical implantation in the rat brain with use of a cylindrical precut die that has a diameter of 3 mm and a depth of 1 mm. Control implants were made in an analogous fashion, but without paclitaxel.

***In Vitro* Release of Paclitaxel.** Paclitaxel release from the Paclimer microspheres was quantified by incubating 10 mg of microspheres in 60 ml of PBS (pH 7.4) at 37°C. Octanol was placed on top of the PBS for continuous extraction of the released Paclitaxel from the PBS. The concentration of paclitaxel in the octanol was assayed by high-performance liquid chromatography.

Cell Lines. Rat 9L gliosarcoma cell lines were grown in DMEM (Life Technologies, Inc.) with 4.5 g/liter glucose, supplemented with 10% fetal bovine serum and penicillin/streptomycin. Tumor allografts were initiated by injecting 1×10^6 cells into the flanks of Fischer 344 rats. Mature tumors were harvested from anesthetized animals and cut into 2-mm pieces on ice for intracranial implantation.

Animal Care. Male Fischer 344 rats weighing 200–225 g were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN), kept in standard animal facilities (4 rats/cage), and given free access to food and water. All surgical procedures were performed under i.p. ketamine/xylazine anesthesia. Animals for necropsy were anesthetized and then perfusion-fixed with 4% phosphate-buffered paraformaldehyde. Rat brains were removed, embedded in paraffin, sectioned, and stained with H&E by the Johns Hopkins tissue biology laboratories.

Surgical Implantation. Tumor pieces and Paclimer discs were implanted in an analogous fashion. A 3-mm burr hole was made in the skull 5 mm posterior and 3 mm to the right of the bregma. The dura was incised sharply, and the underlying cortex was resected with light suction. Hemostasis was obtained by light compression using sterile gauze, and the wound was subsequently irrigated. Dissected pieces of 9L tumor or Paclimer:PEG-1000 discs were implanted in the resection cavity, and the wound was closed with surgical clips (Clay Adams, Parsippany, NJ). For animals receiving both 9L tumor implants and Paclimer:PEG-1000, the tumor piece was implanted on day 0 as described, and the wound was closed. On day 5, the animal was reanesthetized, the clips were removed, and the Paclimer:PEG-1000 implant was placed on top of the tumor implant. The wound was then reclosed with surgical clips. Animals treated with systemic Paclimer were treated on day 5 with an i.p. injection of microspheres (5 mg) in a saline carrier. Survival studies were analyzed by the nonparametric Mantel-Cox method with SPSS software (Chicago, IL).

***In Vivo* Biodistribution.** Paclimer:PEG-1000 implants were prepared as described above, except that [^{3}H]paclitaxel was incorporated into the microencapsulation process. The final specific activity of [^{3}H]paclitaxel in the Paclimer:PEG-1000 implant was 1.2 μCi/mg. Rats receiving labeled implants were euthanized in groups of four at 7 and 30 days after implant. The brains were removed and snap frozen in heptane over dry ice. Any gross residual Paclimer:PEG-1000 implant was removed. The frozen brains were divided into implant and contralateral hemispheres and sectioned coronally at 2-mm intervals. Each section was weighed, dissolved in Solvable homogenizing solution, and combined with Atomlight scintillation mixture (both from New England Nuclear DuPont). The samples were counted on a Beckman LS 6500 liquid scintillation counter. To convert cpm/mg tissue to paclitaxel concentration, a section of tissue adjacent to the polymer was minced and extracted with ethanol. The extract was run on a silica TLC plate (Sigma, St. Louis, MO) spotted with cold paclitaxel and developed in an iodine chamber. The percentage of signal migrating with intact paclitaxel was determined by counting sections of the plate in a scintillation counter.

RESULTS

***In Vitro* Release of Paclitaxel from Polilactofate.** Paclimer was formulated by incorporating paclitaxel into the polilactofate polymer microsphere matrix at a final loading of 10% paclitaxel:90% polilactofate by weight. Using high-performance liquid chromatography, we were able to measure a nearly constant rate of paclitaxel release from the microspheres for 90 days in solution (Fig. 1). Because we wished to measure the distribution of paclitaxel from the microspheres in the rat brain, which has a limited volume, making injection of a microsphere bead slurry impossible, we also combined microspheres with various amounts of polyethylene glycol (M_r 1000; PEG-1000). PEG-1000 is solid at room temperature but liquid at 37°C. We were thus able to model an injection by implanting solid Paclimer:PEG-1000 discs that rapidly liquify to free Paclimer microspheres once implanted. Incorporation of PEG-1000 at ratios of 50% or 75% to Paclimer slightly reduced the initial burst release of paclitaxel compared with free microspheres *in vitro*, but it did not change the slope of the release curve at later time points. Conversely, compressing Paclimer microspheres into discs without PEG-1000 markedly slowed the release rate of paclitaxel, indicating that the increased surface area of free microspheres is critical for optimal paclitaxel release.

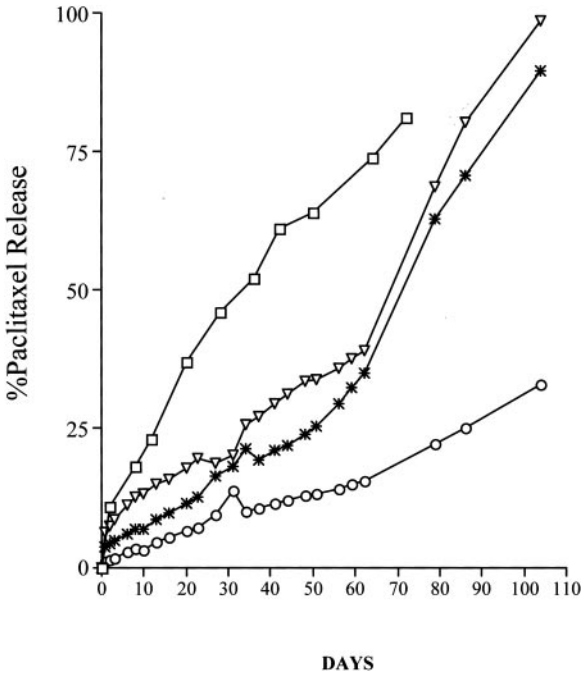

Fig. 1 *In vitro* release of paclitaxel from polilactofate microspheres (Paclimer). Microspheres (10 mg) were loaded with 10% paclitaxel by weight in PBS at 37°C. Release was measured from free microspheres (□) and microspheres compressed into discs with 0% (○), 50% (∗), and 75% (▽) PEG-1000 as an excipient. Results are expressed as cumulative total release as a percentage of initial loading over time.

Biocompatibility of Polilactofate in the CNS. Polilactofate microspheres were implanted in the cerebral hemispheres of adult male rats via open craniotomy. Examination of brains from rats sacrificed by perfusion fixation at 7, 14, and 30 days and 12 weeks after implantation revealed a stereotypic acute neutrophil response, followed by a more chronic macrophage response (Fig. 2*A*). At more extended time points, giant cells became evident, consistent with foreign body reaction and polymer degradation (Fig. 2*B*). The severity of the immune response at each time point was compared with that elicited by PCPP-SA, which is used clinically as an intracranial implant (Fig. 2, *C* and *D*). In each case, the reaction elicited by the polilactofate implants was judged by a blinded neuropathologist (T. T.) to be comparable with or less severe than that elicited by PCPP-SA. Additionally, rats were observed daily for feeding and grooming behavior and weighed at repeated intervals. No behavioral or systemic differences between implant and control animals were noted.

Paclitaxel Toxicity in the CNS. The addition of paclitaxel to the polilactofate microspheres did not produce any additional overt toxicity. No Paclimer-bearing rats showed any evidence of CNS-related toxicity in terms of mortality, behavior, feeding, grooming, or growth. These results are in contrast to results we have reported previously with paclitaxel released from the PCPP-SA matrix, where 25% of rats receiving a PCPP-SA:paclitaxel implant (20–40% paclitaxel by weight) showed significant adverse effects that resulted in death (15). Toxicity was related to the biphasic manner in which the PCPP-SA matrix released the paclitaxel. A significant initial burst of paclitaxel release, of up to 50% of the initial loading, is delivered within 24 h of implantation, followed by much slower controlled release (15). The polilactofate matrix eliminates this burst phase and more closely approximates true zero order kinetics, minimizing random toxicity. Although the overall concentration of paclitaxel in the Paclimer microspheres is slightly lower than that of the PCPP-SA:paclitaxel formulation, we have never seen any toxicity or mortality associated with Paclimer.

Although there were no overt signs of paclitaxel toxicity, histological changes associated with paclitaxel were evident. Macrophages invading the implant site were often seen in states of arrested mitosis and early apoptosis in animals bearing the Paclimer implant (Fig. 2, *E* and *F*), whereas macrophages at the implant site of animals bearing empty polilactofate spheres lacked these histological changes. Moreover, the changes were striking up to 12 weeks after implantation, indicating that biologically active paclitaxel levels were present near the implant site for extended periods. Neuronal architecture became less abnormal more distant from the implant site, although apoptotic cells were still evident. These changes were also observed in animals treated with paclitaxel administered from a PCPP-SA matrix.

***In Vivo* Biodistribution of Paclitaxel Released from Polilactofate.** [^{3}H]Paclitaxel was incorporated into polilactofate:PEG-1000 microspheres and implanted intracerebrally in rats via an open craniectomy. Paclitaxel levels were measured at various distances from the implant by scintillation counting of tissue homogenates and verified as intact paclitaxel by TLC. At both 7 and 30 days after implantation, paclitaxel levels were detectable throughout the rat brain at levels greater than 1 ng/mg brain tissue (approximately 1 nM), with higher levels detectable at 30 days *versus* 7 days (Fig. 3). In comparison, we reported previously that the LD_{90} for paclitaxel *versus* several human glioma cell lines *in vitro* was between 5 and 10 nM (13). By use of this cutoff, therapeutic levels of paclitaxel are achieved 5–7 mm from the implant site at 30 days after implantation.

At each time point, >90% of raw counts represented intact paclitaxel. The assay could not distinguish between free and tissue-bound paclitaxel, so it is likely that a large portion of the drug detected was protein-bound, given that systemically administered paclitaxel is tissue-bound. In a sense, however, the protein-bound fraction serves as an extended controlled-release depot throughout the brain because the protein-bound fraction must exist in equilibrium with free paclitaxel. Histological changes typical of paclitaxel exposure, including arrested mitotic spindles and apoptotic cells, were evident in the brains of rats containing intracranial Paclimer implants up to 12 weeks after implantation, additional evidence that biologically relevant paclitaxel levels exist in the brain for extended times after implantation (Fig. 2*F*).

Efficacy of Paclimer against Glioma. Rats bearing established 9L glial tumors were treated with Paclimer implants. The intracranial 9L glioma model was selected for study because it previously predicted the eventual success of PCPP-SA polymers loaded with BCNU (Gliadel) in Phase III clinical trials (2, 16). In our experiments, Paclimer doubled the median survival of rats bearing tumors (35 days *versus* 16 days; $n = 10$ animals/group; $P < 0.0001$, Kaplan-Meier Method) compared

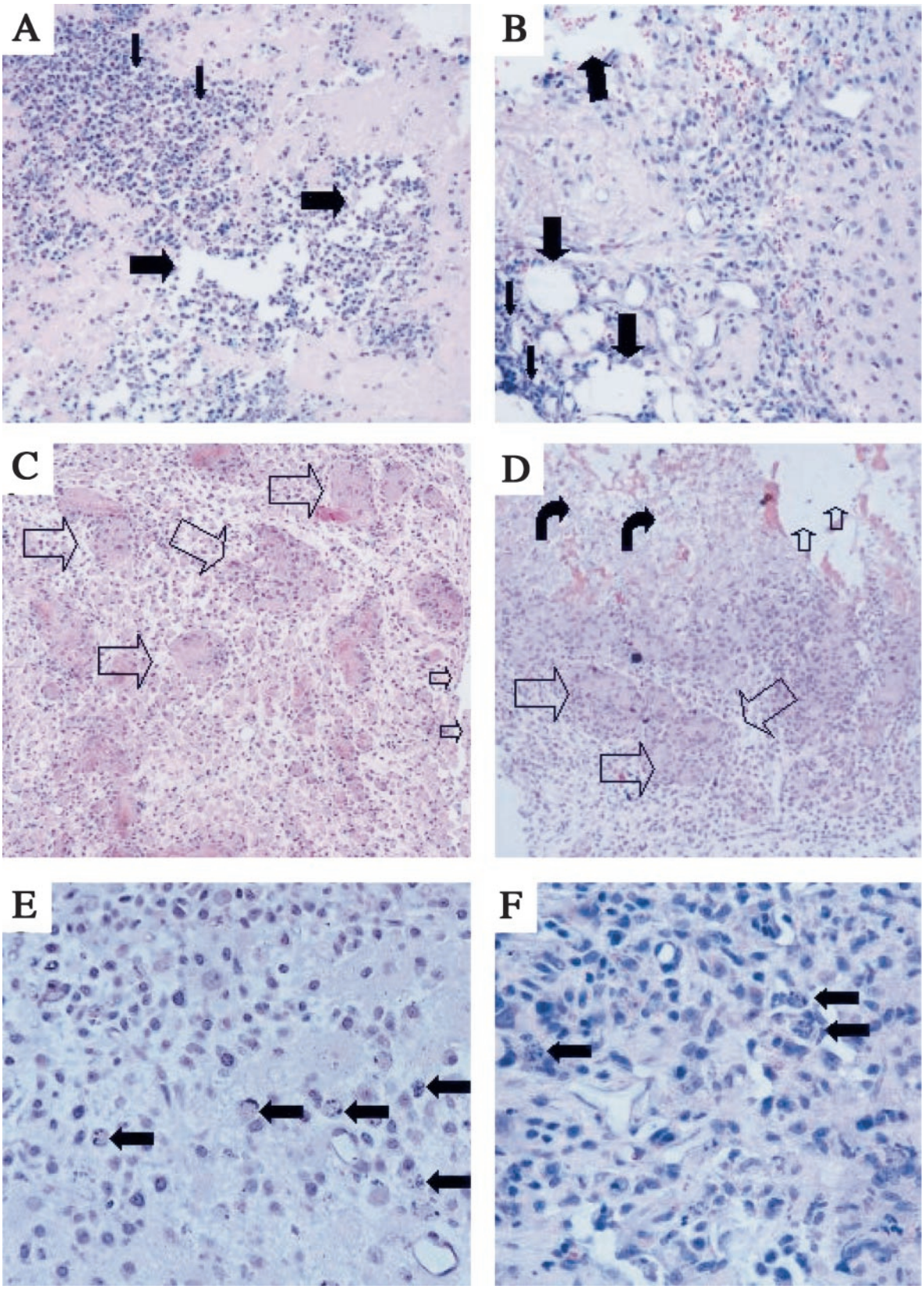

Fig. 2 Comparison of inflammatory reaction elicited by empty polilactofate microspheres and PCPP-SA implanted in the rat brain. H&E-stained sections (×100 magnification) of perfusion-fixed rat parietal lobe 7 days after implantation for (*A*) polilactofate and (*B*) PCPP-SA demonstrate a polymorphonuclear infiltrate (*small closed arrows*) adjacent to void spaces corresponding to the sites of polymer implantation (*large closed arrows*).

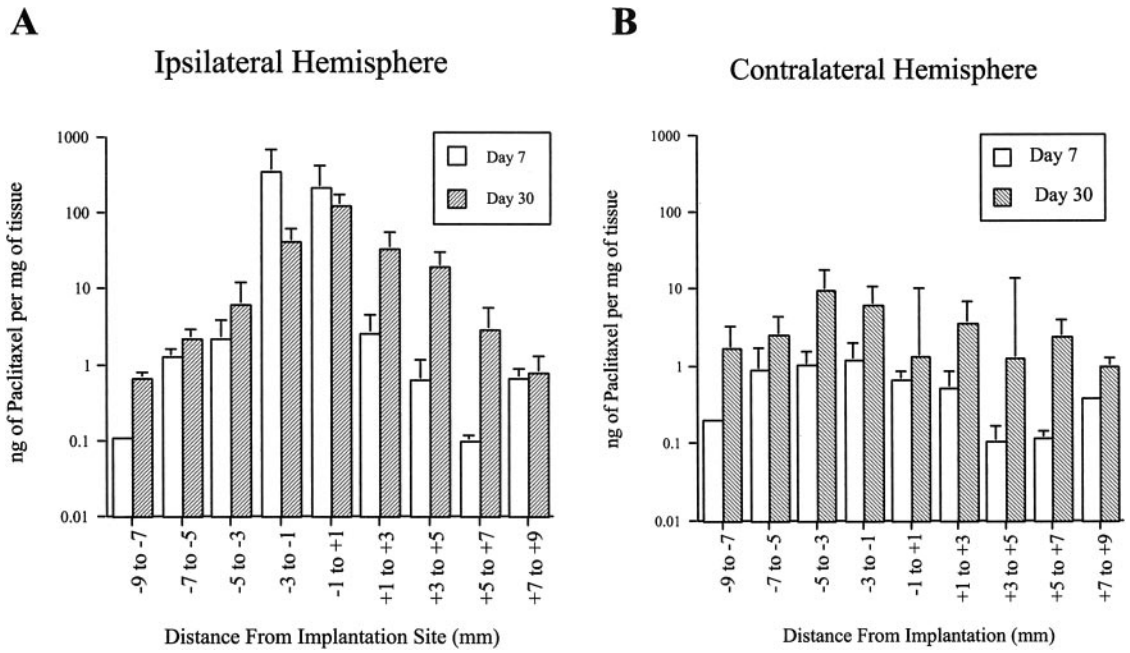

Fig. 3 *In vivo* biodistribution of paclitaxel delivered from polilactofate microspheres in the rat brain. [^{3}H]Paclitaxel was incorporated into polilactofate microspheres (Paclimer) at 10% loading and compressed into 10-mg discs containing 50% PEG-1000 by weight for implantation in the rat parietal lobe. Brains were removed at 7 and 30 days after implantation and divided into implant (*A*) and contralateral (*B*) hemispheres. Each hemisphere was sectioned at 2-mm intervals in the coronal plane, and the amount of paclitaxel present in the section was quantitated by liquid scintillation counting. Measurements represent mean of three separate animals ± SE.

with survival in control animals (Fig. 4). All of the animals died with large tumors, as verified at necropsy. Empty microspheres had no effect on survival compared with animals receiving no treatment, indicating that survival differences were due to paclitaxel release from the matrix. Additionally, an equivalent dose of Paclimer delivered systemically had no effect on survival, indicating that local administration of Paclimer was critical to efficacy. Each experiment also produced rats treated with Paclimer that were long-term survivors with no tumor evident at the time of sacrifice 90 days after implantation.

DISCUSSION

Devising a successful method for a drug to bypass the blood-brain barrier has the potential to transform an ineffective compound into an important advance in patient care. At present, only one biodegradable polymer construct, PCPP-SA, is United States Food and Drug Administration-approved for drug delivery to the brain. PCPP-SA is not optimal for the delivery of all possible pharmaceutical compounds, however. In our previous report (15), we examined paclitaxel delivery via PCPP-SA implants in rodent models of malignant brain tumors and demonstrated that whereas the implant was efficacious against brain tumors, the matrix released drug in a biphasic pattern with an initial burst of drug followed by a much slower release of drug over months. The burst of paclitaxel release resulted in sporadic toxicity among animals implanted with the PCPP-SA:paclitaxel matrix. A similar biphasic release was seen by investigators using poly(ϵ-caprolactone) and poly(ethylene oxide)-poly(lactide/glycolide) controlled release polymers in non-CNS applications (17, 18).

In contrast, polilactofate microspheres were able to release paclitaxel *in vitro* at a constant rate for the entire period of drug release. Correspondingly, the sporadic toxicity associated with the unpredictable burst phase of drug release was not observed in our model. At the same time, the immune reaction elicited by the polilactofate polymer backbone was comparable with or less pronounced than that caused by PCPP-SA, indicating that polilactofate should be biocompatible in the CNS. Minimizing host

The severity of the polymorphonuclear reaction was more profound and extended a greater distance from the implant site in the PCPP-SA-treated animals than in the polilactofate-treated animals. H&E-stained sections (×40 magnification) of parietal lobe at 30 days after implantation in (*C*) polilactofate- and (*D*) PCPP-SA-treated animals demonstrated a giant cell reaction (*large open arrows*) adjacent to the implanted polymer (*small open arrow*). The degree of foreign body reaction was comparable. Areas of necrosis were present adjacent to all PCPP-SA implants (*curved arrows*) but absent from polilactofate implants. H&E-stained sections (×400 magnification) of perfusion-fixed rat parietal lobe from animals receiving polilactofate implants loaded with paclitaxel (Paclimer) at (*E*) 7 and (*F*) 84 days after implantation demonstrate abundant cells in arrested mitosis/early apoptosis, consistent with paclitaxel exposure (*large arrows*). A background of reactive astrocytosis and a moderate increase in vascularity are apparent at both time points.

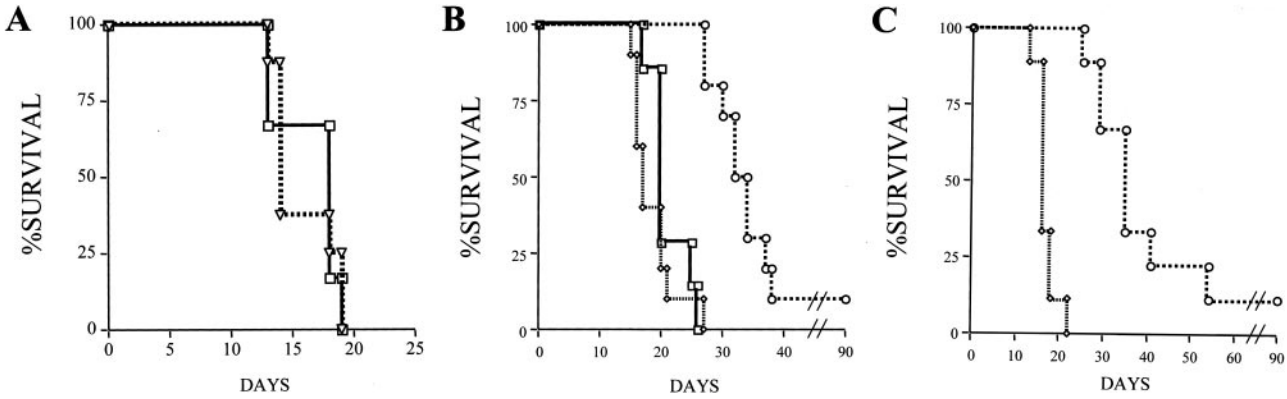

Fig. 4 Kaplan-Meier survival curves of rats bearing established intracranial 9L gliosarcomas tumors treated with 10-mg Paclimer:PEG-1000 (50:50) implants (○). *A,* systemically administered i.p. Paclimer (▽) did not prolong survival compared with animals receiving no treatment (□). Median survival of i.p. Paclimer-treated animals = 14 days, median survival of control-treated animals = 18 days ($P = 0.0944$). *B,* Paclimer (○) implanted intratumorally extended median survival compared with animals receiving no treatment (□). Median survival of Paclimer-treated animals = 33 days, median survival of control-treated animals = 20 days ($P < 0.0001$). Blank polilactofate:PEG-1000 microspheres (◇). Median survival of Paclimer-treated animals = 33 days, median survival of polilactofate-treated animals = 17 days ($P < 0.0001$). *C,* Paclimer (○) extended median survival compared with control groups receiving empty polilactofate:PEG-1000 microspheres (◇). Median survival of Paclimer-treated animals = 35 days, median survival of polilactofate-treated animals = 16 days ($P < 0.001$). Statistical analysis was performed using the nonparametric Mantel-Cox method with SPSS software.

reaction to an intracranial implant is critical in preventing long-term clinical sequelae from the implant, such as gliosis, seizures, or neurological deficit. None of the rats receiving polilactofate implants had any of these complications.

In addition to providing controlled release of drug, a matrix must protect incorporated drug from degradation before release. At 30 days after implantation, we determined that >90% of drug within the brain corresponded to intact paclitaxel. Furthermore, histological changes typical of acute paclitaxel exposure were evident in the brains of rats receiving Paclimer implants up to 12 weeks after implantation, consistent with ongoing exposure of the brain to active drug.

The clinical possibilities of polilactofate-based drug delivery are profound. Paclitaxel shows cytotoxic effects in clonogenic, cytotoxic, and cell growth assays *versus* brain tumors at nanomolar concentrations (12–14). Additionally, several reports demonstrate a theoretical radiosensitizing effect of paclitaxel, given its ability to arrest cells at the G_2-M transition of the cell cycle (12, 19). Unfortunately, several Phase II clinical trials of paclitaxel *versus* glioma have failed to demonstrate a conclusive effect of paclitaxel *versus* malignant brain tumors (4–10). Phase I pharmacokinetic data demonstrate that paclitaxel is not detectable within the CNS of patients 2 h after i.v. infusion (4, 11). In contrast, polilactofate maintained therapeutic paclitaxel levels within the CNS of rodents for 30 days. Locally implanted Paclimer is correspondingly able to inhibit brain tumor growth in brain tumor models when systemically administered paclitaxel is not. It is reasonable to hypothesize that Paclimer may be similarly able to boost drug levels in the clinical setting and produce therapeutic responses that were not seen with i.v. infusions of paclitaxel. Polilactofate microspheres, therefore, have the potential to broaden the spectrum of drugs that may be considered useful for diseases of the CNS.

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