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J Neurooncol. Author manuscript; available in PMC 2006 November 7.

Published in final edited form as:
J Neurooncol. 2006 January ; 76(2): 131–138.

Local Intracerebral Administration of Paclitaxel with the Paclimer® Delivery System: Toxicity Study in a Canine Model

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Abstract

Introduction: Paclitaxel, a microtubule binding agent with potent anti-glioma activity *in vitro*, exhibits poor penetrance to the CNS when delivered systemically. To minimize toxicity and reach therapeutic concentrations in the CNS, paclitaxel was previously incorporated into biodegradable microspheres (Paclimer®), and the efficacy of Paclimer® was determined in a rat model of malignant glioma. In this study we report the safety of intracranial Paclimer® in a canine dose escalation toxicity study to prepare its translation into clinical scenarios.

Methods: Twelve normal beagle dogs underwent a right parieto-occipital craniectomy and were randomized to receive either Paclimer® at 2-mg/kg (n=5), empty microspheres at 2-mg/kg (n=1), Paclimer® at 20-mg/kg (n=5), or empty microspheres at 20-mg/kg (n=1). Post-operatively, dogs were observed daily for signs of neurotoxicity. Complete blood counts and plasma levels of paclitaxel were obtained weekly. CSF levels and MRI scans were obtained on days14-120. Paclitaxel concentrations were quantified by LC-MS.

Results: Animals treated with 20-mg/kg Paclimer® had minimal paclitaxel levels in plasma (range 0-7.84 ng/ml) and CSF (range 0-1.16 ng/ml). Animals treated with 2mg/kg Paclimer® had undetectable levels of paclitaxel in plasma, CSF was not obtained to minimize animal suffering. All animals exhibited normal behavior and weight gain, and were alive post-operatively through the last day of the study (day 60-120) without signs of neurological toxicity. There was no evidence of systemic toxicity or myelosuppression. MR imaging was comparable between Paclimer® animals and controls. Adverse effects included wound infections and a brain abscess, all of which responded to antibiotic therapy, and one ventriculomegaly due to communicating hydrocephalus.

Conclusions: Paclimer®-based delivery of paclitaxel is safe for intraparenchymal delivery at the tested doses in normal dogs.

Keywords

Paclimer; paclitaxel; taxol, microspheres; brain tumor; glioma; dogs; toxicity; safety

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Disclosures:

Under separate licensing agreements between the Johns Hopkins University and Guilford Pharmaceuticals, Inc. and the Johns Hopkins University and Angiotech Pharmaceuticals, Inc., Dr. Brem is entitled to a share of royalty received by the University on sales of products described in this article. Dr. Brem owns Guilford Pharmaceuticals, Inc. stock, which is subject to certain restrictions under University policy. Dr. Brem also is a paid consultant to Guilford Pharmaceuticals, Inc. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

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Introduction:

Despite significant advances in diagnostic modalities and therapeutic interventions, the prognosis for patients with malignant gliomas remains poor (1,2). The restricted permeability of the blood-brain barrier (BBB) permits only the entry of small hydrophobic molecules, a limited set of actively transported molecules such as glucose and particular amino-acids, and some macromolecules (3). Ideal drug candidates tend to be smaller molecules with simple structures, increased plasma availability, and high lipid solubility (4,5). These characteristics limit the spectrum of effective antineoplastic agents that can achieve interstitial therapeutic levels in the central nervous system (CNS) with acceptable systemic toxicity (3,6,7).

The development of controlled-release polymers for local delivery of drugs to the brain bypasses the limitations of the BBB, minimizes systemic toxicity, and enhances local concentrations of the agents delivered (3,6). Biodegradable controlled-release polymers loaded with carmustine have been established as a safe and effective therapy for patients with malignant gliomas (8-12). Given the inherent advantages of locally-delivered chemotherapy, there has been continued interest in the development of additional controlled-release drug delivery systems suitable for intraparenchymal administration of novel antineoplastic compounds.

Paclitaxel is a microtubule-binding agent that has proven to be clinically efficacious for the treatment of various human neoplasias including ovarian and breast cancers when administered systemically (13-18). Paclitaxel exhibits potent cytotoxic activity against malignant gliomas *in vitro*, but has failed to prolong survival of brain tumor patients in clinical trials when administered systemically (19-21). Systemic administration of paclitaxel also induces a dose-dependent toxicity that ranges from sensory neuropathy and gastrointestinal disturbances to severe myelosuppression (22-25). The lack of efficacy observed with systemic paclitaxel in patients with brain tumors could be attributed to the low permeability of the BBB to paclitaxel and the resultant inadequate bioavailability of paclitaxel in the CNS at maximally tolerated systemic doses of the drug (26-28).

The results of a phase II study of locally-delivered paclitaxel using convection-enhanced drug delivery (CEDD) for treatment of patients with recurrent glioblastoma multiforme were recently published and showed that paclitaxel administered via CEDD had marked anti-tumor responses but generated a significant incidence of treatment-associated complications (29). The delivery of paclitaxel via CEDD and its effect within the tumor can be monitored clinically usingdiffusion-weighted magnetic resonance imaging (DW-MRI)(30). In this study paclitaxel was administered to 15 patients, 3 patients received a dose of 7.2 mg/day and the remaining 12 patients received a dose of 3.6 mg/day. Complications reported by the authors included chemical and bacterial meningitis, ventriculomegaly, subdural empyema, poor wound healing, and increased peri-tumoral edema. These studies are encouraging and validate the use of local delivery for intratumoral paclitaxel administration.

In order to achieve local sustained administration of paclitaxel into the CNS, we had previously incorporated paclitaxel into a biodegradable polymer matrix consisting of a polyphosphoester polymer (polilactofate) in a microsphere form with a loading concentration of 10% paclitaxel by weight (Paclimer® delivery system)(27). In that study we found that Paclimer® was capable of delivering paclitaxel in a sustained fashion for up to 90 days *in vitro*. There was no local or systemic toxicity in the rat brain and Paclimer®-delivered paclitaxel prolonged the survival of rats challenged intracranially with 9L gliosarcoma (27).

The results of these preclinical studies further support the role of local-delivery of paclitaxel in brain tumor therapy and confirm the need for a safer and more effective system for

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intracranial paclitaxel delivery. Therefore we conducted the present study to establish the safety of intracerebral Paclimer® when administered at doses of 2 and 20 mg/kg in the dog brain.

Materials and methods:

Formulation of paclitaxel microspheres

The Paclimer® delivery system, a controlled release formulation of paclitaxel, was obtained from Guilford Pharmaceuticals, Inc. (Baltimore, MD). We have previously described the technique for preparation of Paclimer®(27). Briefly, Paclimer® was synthesized by incorporating paclitaxel into the bioerodable polyphosphoester (PPE) polymer, p(DAPG-EOP), in the form of microspheres at 10% loading by weight. Both substances were dissolved in ethyl acetate and the resulting solution was pumped through an in-line homogenizer with 0.5% polyvinyl acetate solution in a container with an overhead stirrer. The microspheres were allowed to harden, and the product obtained was filtered and lyophilized. Control empty PPE microspheres were synthesized in a similar fashion.

Animals

Twelve normal adult male beagle dogs weighing 12-14 kg were obtained from Harlan Inc. (Indianapolis, IN) and housed individually with free access to Baltimore city water and dog chow. All animal protocols were approved by the Animal Care and Use Committee of The Johns Hopkins University School of Medicine.

Experimental design

Animals (n=12) were assigned to two experimental groups. Dogs in the first group (n=6) were surgically implanted with either intracerebral Paclimer® at a dose of 20 mg/kg (n=5) or with the equivalent amount of empty microspheres (n=1). Dogs in the second group (n=6) were surgically implanted with either intracerebral Paclimer® at a dose of 2 mg/kg (n=5), or with the equivalent amount of empty microspheres (n=1). All animals were evaluated daily for signs of neurological or systemic toxicity, and sacrificed at pre-determined time points for complete pathological analysis. Animals in the 20 mg/kg Paclimer® group were sacrificed in a staggered fashion on post-operative days (POD) 59 (n=1), 62 (n=1), 99 (n=1), 101 (n=1), and 125 (n=2). All animals in the 2 mg/kg Paclimer® group were sacrificed on POD 120-123 (Table 1).

Surgical technique

The dogs were sedated with an intramuscular (IM) injection of acepromazine and transferred to the operating suite. An intravenous (I.V.) line was started for continuous infusion of Lactated Ringer's solution and general anesthesia was induced with I.V. sodium thiopental. The animals were intubated endotracheally for mechanical ventilation. General anesthesia was maintained using inhalation isofluorane with continuous cardiac and respiratory monitoring. Each dog was positioned prone and the scalp over the operative site was shaved, disinfected, and draped in sterile fashion. A small parasagittal incision was made over the right parieto-occipital bone and scalp bleeding was controlled with electrocautery. The underlying connective tissue and muscle were divided with electrocautery, and a high-speed air drill was used to create a 2.0-cm parieto-occipital craniectomy. A 1.5-cm dural incision was made to expose the underlying parieto-occipital cortex, and a 1.5-cm corticotomy was made using bipolar cautery and gentle suction. Once hemostasis was achieved, sterile Paclimer® or empty microspheres were placed intraparenchymally and secured by placing a rectangular piece of Surgicel® over the corticotomy site. The surgical field was irrigated and a rectangular piece of Gelfoam® was placed to fit the craniectomy defect. The incision was closed in a layered fashion using interrupted 3.0 Vicryl and running 3.0 Prolene® sutures. After discontinuation of anesthesia, the animals were extubated uneventfully and transferred to the housing facility for observation.

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Blood and CSF Sampling and Analysis

Blood was collected from peripheral veins pre-operatively, as well as weekly during the post-operative period in all animals. The blood samples were analyzed by an automated cell counter for complete blood count and white blood cell (WBC) differential. Animals implanted with 20 mg/kg Paclimer® underwent lumbar or cisternal punctures for collection of cerebrospinal fluid (CSF) on POD 14, 44, 59, 61, 99, 101, and 125 and Gram staining, cell counts, protein concentration, and glucose levels were performed. The blood and CSF samples were also assayed for paclitaxel concentration by liquid chromatography mass spectrometry (LC-MS). In animals implanted with 2 mg/kg Paclimer® CSF was not collected and Paclimer® levels were not established to minimize animal suffering since the levels of paclitaxel obtained from the animals treated with the 20 mg/kg dose were minimal.

Radiographic studies

In weeks 2, 6, 8, and 18 post-implantation, the animals were sedated, anesthetized, and intubated as described previously, and magnetic resonance imaging (MRI) with and without gadolinium contrast enhancement was conducted.

Necropsy and Histology

All animals were sacrificed at the scheduled time points. Before sacrifice, blood and CSF were obtained as described above. Complete necropsies were performed on all animals by a veterinary pathologist and the brain, the meninges, and other organs were fixed in 10% paraformaldehyde and embedded in paraffin for histopathological examination.

Results:

Survival, toxicity, and adverse events

In the 20 mg/kg Paclimer® group, the control animal and four out of five dogs that received the intracranial Paclimer® microspheres survived to the scheduled time points of 30, 60, 90, and 120 post-operative days, and exhibited no signs of neurological or systemic adverse events (Table 1). Two of the animals developed wound infections and partial incisional dehiscence that promptly responded to wound irrigation and a prophylactic seven day course of I.M. ceftriaxone. On POD 56, one of the treatment animals developed signs of increased intracranial pressure (i.e. somnolence, bradycardia) that were secondary to a communicating hydrocephalus, which was initially managed by a large-volume CSF drainage; however, because of ethical considerations, the animal was euthanized on POD 59, one day before his scheduled sacrifice date of POD 60. This complication was likely caused by accidental intraventricular administration of Paclimer®.

In the 2 mg/kg Paclimer® group, the control animal and all five dogs that received the intracranial Paclimer® microspheres survived to their scheduled time points of 120 days (Table 1). One animal implanted with Paclimer® developed transient gait disturbances and signs of meningismus on POD 37. MRI showed a deep brain abscess with significant edema and mass effect that was successfully treated with 4 weeks of I.M. ceftriaxone (Figure 1). The neurological exam of this animal returned to baseline after treatment. Two treatment animals developed wound infections or partial incisional dehiscence that responded to wound irrigation and a prophylactic seven day course of IM ceftriaxone.

Throughout the study period, animals in both groups were weighed at weekly intervals. Animals receiving blank microspheres and those receiving Paclimer® displayed appropriate weight gain regardless of the dose administered.

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Myelosuppression following systemic paclitaxel administration is a serious dose-limiting toxicity (25). When compared to the control animal, the animals receiving intracranial Paclimer® at 20 mg/kg did not develop leukopenia (Figure 2-A), anemia (Figure 2-B), or thrombocytopenia (Figure 2-C) regardless of the dose administered. Animals treated with 2 mg/kg Paclimer® or control microsphere also had unremarkable hematological values throughout the study.

Systemic and CSF concentrations of paclitaxel

Animals in the 20 mg/kg Paclimer® group had minimal amounts of paclitaxel in plasma (range 0-4.08 ng/ml) (Figure 3-A). Likewise, the concentration of paclitaxel in the CSF was either minimal or below the level of quantification (<0.5 ng/ml) (Figure 3-B). These measurements were not obtained in the 2 mg/kg Paclimer® group to minimize the number of invasive procedures in the animals.

Biocompatibility of Paclimer® in the CNS

The response of the brain parenchyma to Paclimer® and blank microspheres at both doses was followed over time using MRI with and without gadolinium enhancement. In the 20 mg/kg Paclimer® group images showed mild edema surrounding the site of implantation that was comparable between control and Paclimer® animals, which resolved by the last day of follow-up (Figure 4). In the 2 mg/kg Paclimer® mild edema surrounding the implantation site was also observed, which resolved by the last day of follow-up.

Detailed necropsy examination of the animals at 120 days for the 2 mg/kg group, and at 60, 100, and 120 days for the 20 mg/kg group, did not reveal any overt signs of organ toxicity attributable to polilactofate microspheres or paclitaxel. A closer inspection of brains of the control and treatment animals showed an area of fibrous adhesion present between the inner table of the calvarium and the implant site (Figure 5).

Histological examination of meningeal and brain specimens obtained after necropsy near the implant site revealed chronic inflammatory reaction and superficial gliosis that was limited to the parenchyma immediately surrounding the implant site. The extent of this cuff of gliosis and chronic inflammatory response was similar in the control and treatment animals (Figure 5). The brain of the Paclimer®-treated dog that developed communicating hydrocephalus displayed marked ventriculomegaly and mild meningeal hyperplasia.

Discussion:

In this study we report the safety of intracranial administration of paclitaxel delivered via the Paclimer® system at 2 and 20 mg/kg in dogs. We found that at a dose of 2 mg/kg of Paclimer®, paclitaxel generates no signs of systemic or neurologic toxicity. When Paclimer® was administered at a dose of 20 mg/kg no signs of systemic toxicity were observed, minimal levels of paclitaxel were detected in plasma and CSF, but a neurologic complication occurred in one of the treated animals. This complication was secondary to accidental intraventricular administration of Paclimer®. Even though this type of complication would be amenable to treatment in clinical settings the animal was euthanized to minimize suffering. All other animals treated with 20 mg/kg had no neurologic complications. Clinical studies using CEDD of paclitaxel showed a similar complication, which was attributed to leakage of paclitaxel into the subarachnoid space (29). Accidental intrathecal administration of other anti-microtubule agents has been shown to produce a wide range of complications and should be avoided (31, 32).

Other treatment-related complications found in the animals included wound dehiscence and wound infection. It has been proposed that this complication is caused by subcutaneous leaking

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of paclitaxel, which interferes with adequate wound healing (29). In our study one animal in every group experienced suture dehiscence, but a complete response was obtained after wound irrigation and antibiotic therapy. Wound infections were present in one animal of the 2 mg/kg group and in two animals in the 20 mg/kg group (including 1 animal treated with blank microspheres), all of these animals responded to antibiotic therapy. One animal in the 2 mg/kg group presented with a deep brain abscess that was successfully treated with antibiotics, this was most likely a complication resulting from surgical contamination.

Radiological evaluation of the animals showed minimal edema surrounding the site of implantation that resolved by the day of sacrifice in both treatment and control animals for both dose groups. Pathological evaluation of the implanted areas 120 days after implantation of Paclimer® in the 2 mg/kg group and 60, 100, and 120 days in the 20 mg/kg showed changes consistent with the surgical manipulation that were comparable between animals treated with Paclimer® at both doses and animals treated with blank microspheres. No abnormalities were found in the surrounding brain parenchyma in any of the animals in the study. The chronic inflammatory response seen around the implantation area tends to decrease over time as the polymer is degraded. By day 125 most of the polymer has been degraded and absorbed and changes compatible with parenchymal healing are present with minimal chronic inflammatory changes.

The toxicity of systemic administration of paclitaxel has been previously determined by Poirier et al in dogs (33). In that study severe myelosuppression was consistently observed in the animals at clinically relevant doses. Intraparenchymal implantation of Paclimer® microspheres did not induce significant myelosuppression in dogs at the doses administered. As shown in Figure 2, a descending trend in WBC counts and platelets was observed in animals treated with 20 mg/kg of Paclimer® towards the end of the study. This decline, however, was not clinically significant. In order to fully determine additional toxicities evaluation of other parameters such as activity of natural-killer lymphocytes, mitogen responses, and phagocytic activity, is warranted and should be considered during clinical testing. Although complications observed in the animals including wound infections, sutures dehiscence, and brain abscess could be related to immune system dysfunction, at the time of presentation, WBC and platelet counts were within normal limits.

In the present study we selected normal canines over lower species in order to evaluate their neurological status in a more detailed fashion. We acknowledge that the effects from implantation of the Paclimer® system in a normal brain could differ from the effects of implantation in tumor-bearing tissue where tumorigenic edema and hypoxia are present. Furthermore, Paclimer® will be tested clinically in patients that are likely to have received both radiation therapy and cytotoxic chemotherapy, which could also alter the diffusion and absorption of paclitaxel.

The pharmacokinetics of Paclimer® have been previously described *in vitro* (27). The polilactofate microspheres used in this study where selected based on the safety and efficacy studies in the rat brain (27). In previous studies by our group, paclitaxel was initially incorporated into a biodegradable polymer matrix consisting of paclitaxel and a polyanhydride poly[1,3-bis (carboxyphenoxy) propane-co-sebacic-acid] (PCPP:SA) polymer. The safety and efficacy of this formulation were also evaluated in a rodent model of malignant glioma (34). In this study a bi-phasic release profile of paclitaxel generated sporadic toxicity, for this reason polilactofate was preferred over PCPP:SA for paclitaxel delivery. The efficacy studies showed that locally delivered Paclimer® doubled the median survival of F344 rats bearing intracranial 9L gliosarcoma (35 days vs. 16 days; n=10 animals/group; $P < 0.001$) compared to controls, and that systemic administration of an equivalent dose of Paclitaxel had no effect on animal survival (27).

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Paclimer® has also demonstrated beneficial effects against human prostate tumors implanted in nude mice (35). Treatment of the xenografts with intratumoral Paclimer® alone significantly decreased tumor size compared to controls, and showed synergistic activity when combined with radiation therapy (35). This effect has also been observed by our group in intracranial 9L gliosarcomas in rats using a combination of Paclimer® and radiation therapy (36). This effect appears to be related to the radiosensitizing properties of paclitaxel (20,37), which depends on stabilization of tubulin formation that creates a block in the G2-M phase of the cell cycle (the most radiosensitive phases)(35).

Paclimer® is currently undergoing clinical testing for recurrent ovarian cancer and the results of a phase I study are being prepared for publication (Gynecologic Oncology Group, Dr. Deborah Armstrong, personal communication).

In summary, Paclimer® has demonstrated potent anti-glioma effects in animal models and can be administered intracranially at doses ranging between 2 and 20mg/kg with acceptable margins of safety within the limitations of this toxicity study.

Acknowledgements:

This work was supported in part by National Cancer Institute grants U19 CA52857 (H.B.), NCI NSRA T32 CA09574 (P.P.W.), and F32 CA83287 (K.A.W.). We would like to thank John Ziewackz and Jonathan Winter for their assistance in this project.

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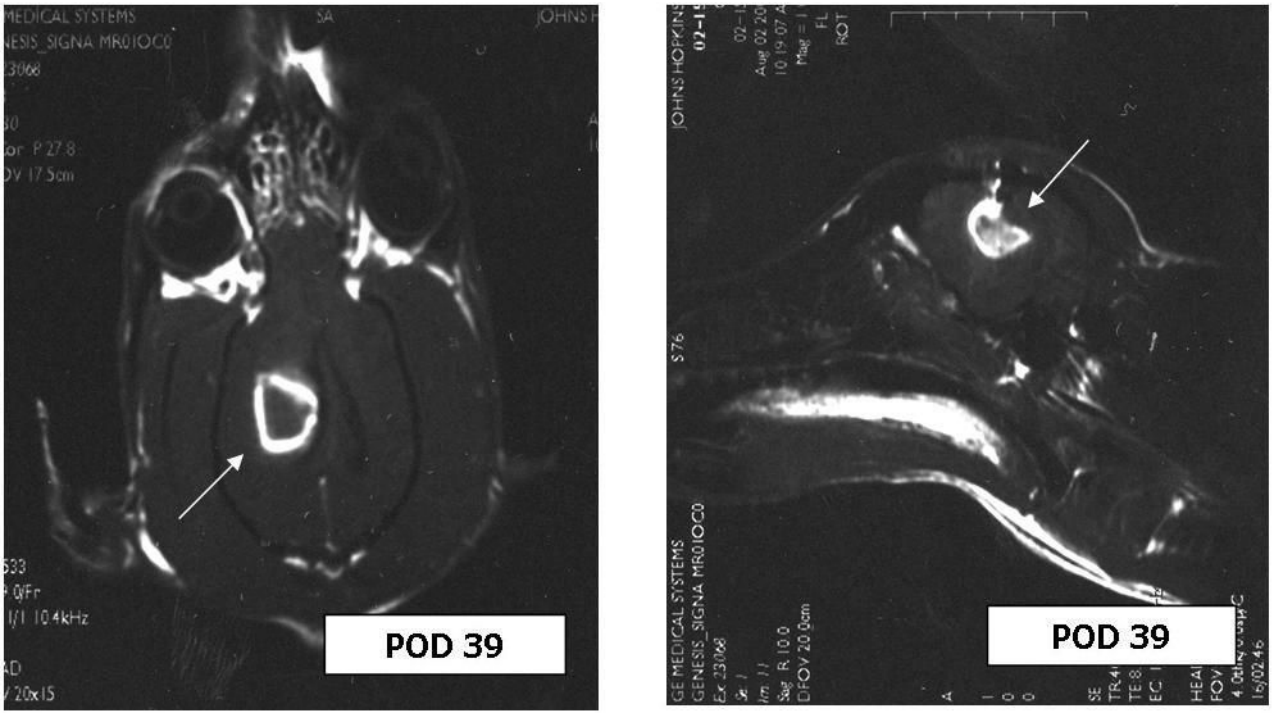

Figure 1.
MRI of an animal treated with 2 mg/kg Paclimer® that presented with a deep brain abscess.

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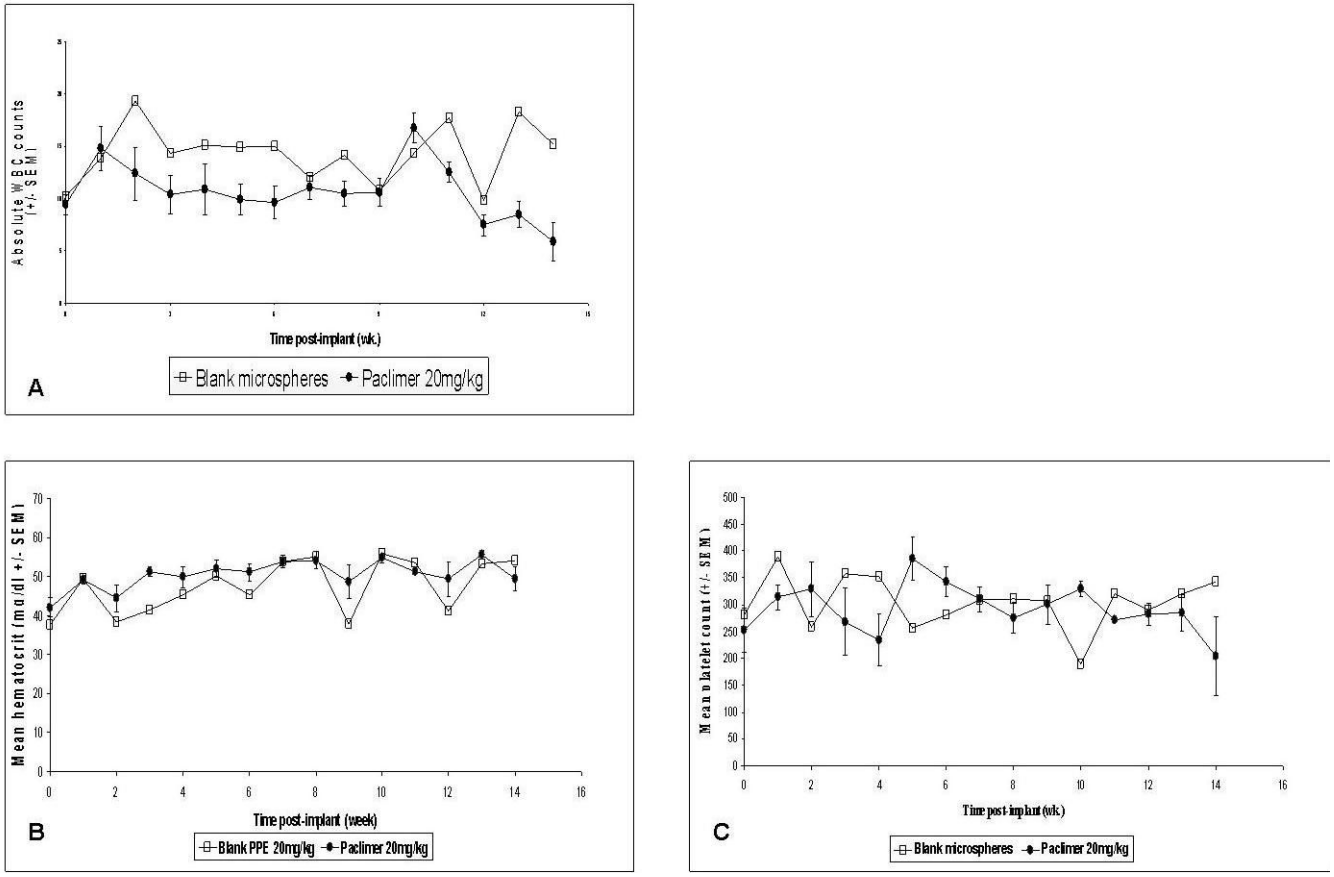

Figure 2.

Panel shows the progression of hematological values in animals treated with 20 mg/kg Paclimer®. Line graphs show the WBC counts (A), hematocrit (B) and platelets counts (C).

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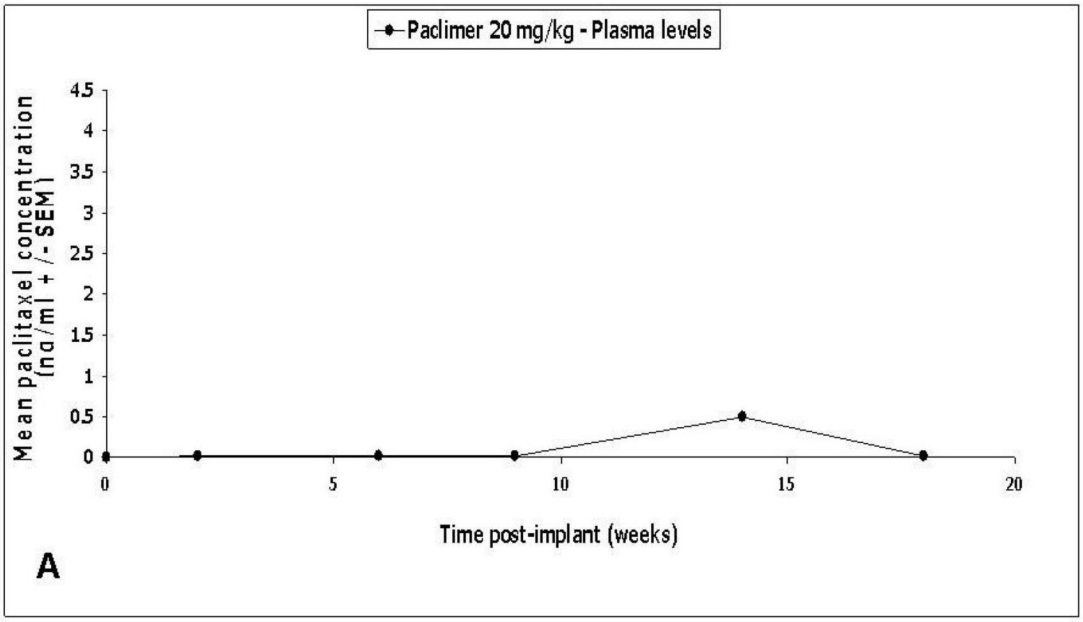

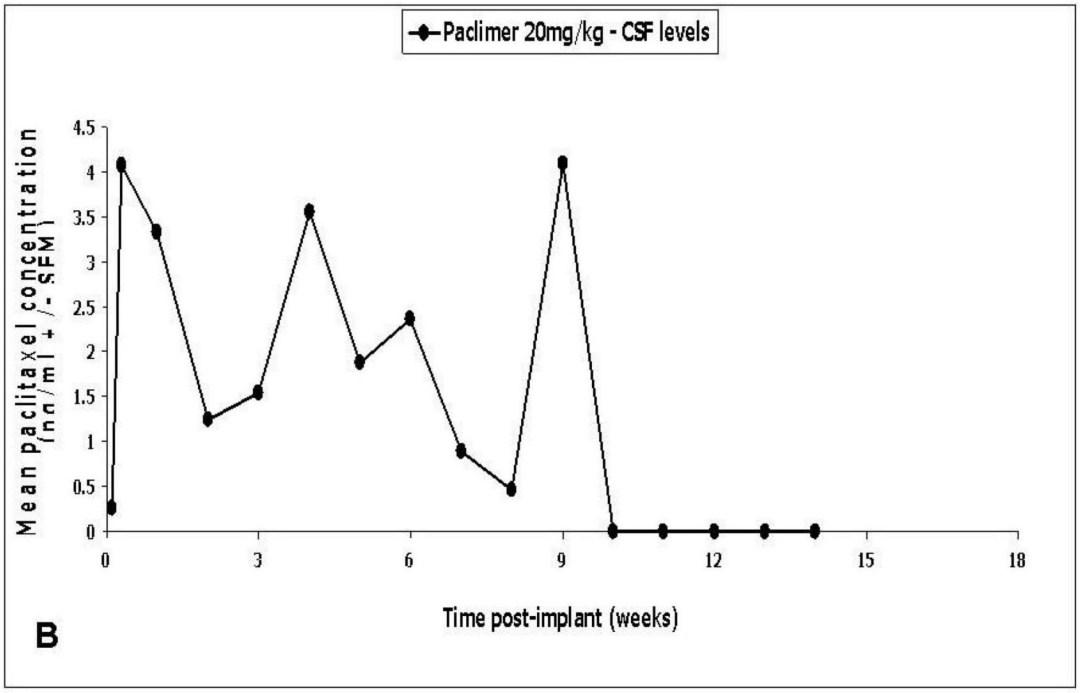

Figure 3.

A. Levels of Paclimer® in plasma from animals treated with 20 mg/kg. **B.** Levels of Paclimer® in CSF from animals treated with 20 mg/kg.

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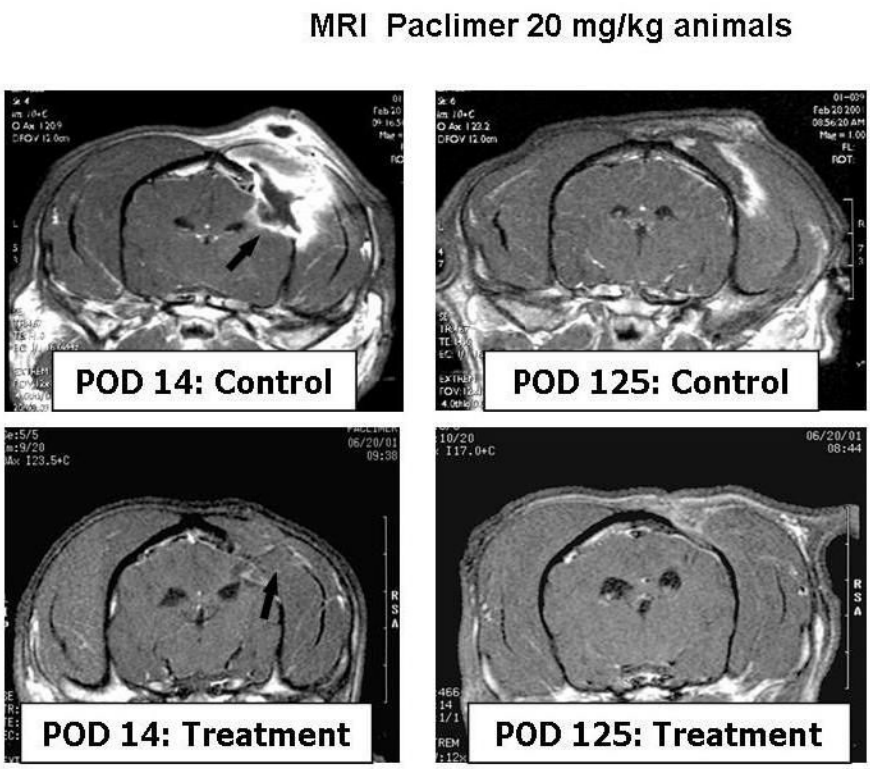

Figure 4.
MRI showing coronal sections of the brain of animals treated with Paclimer® 20 mg/kg (bottom) and blank microspheres (top).

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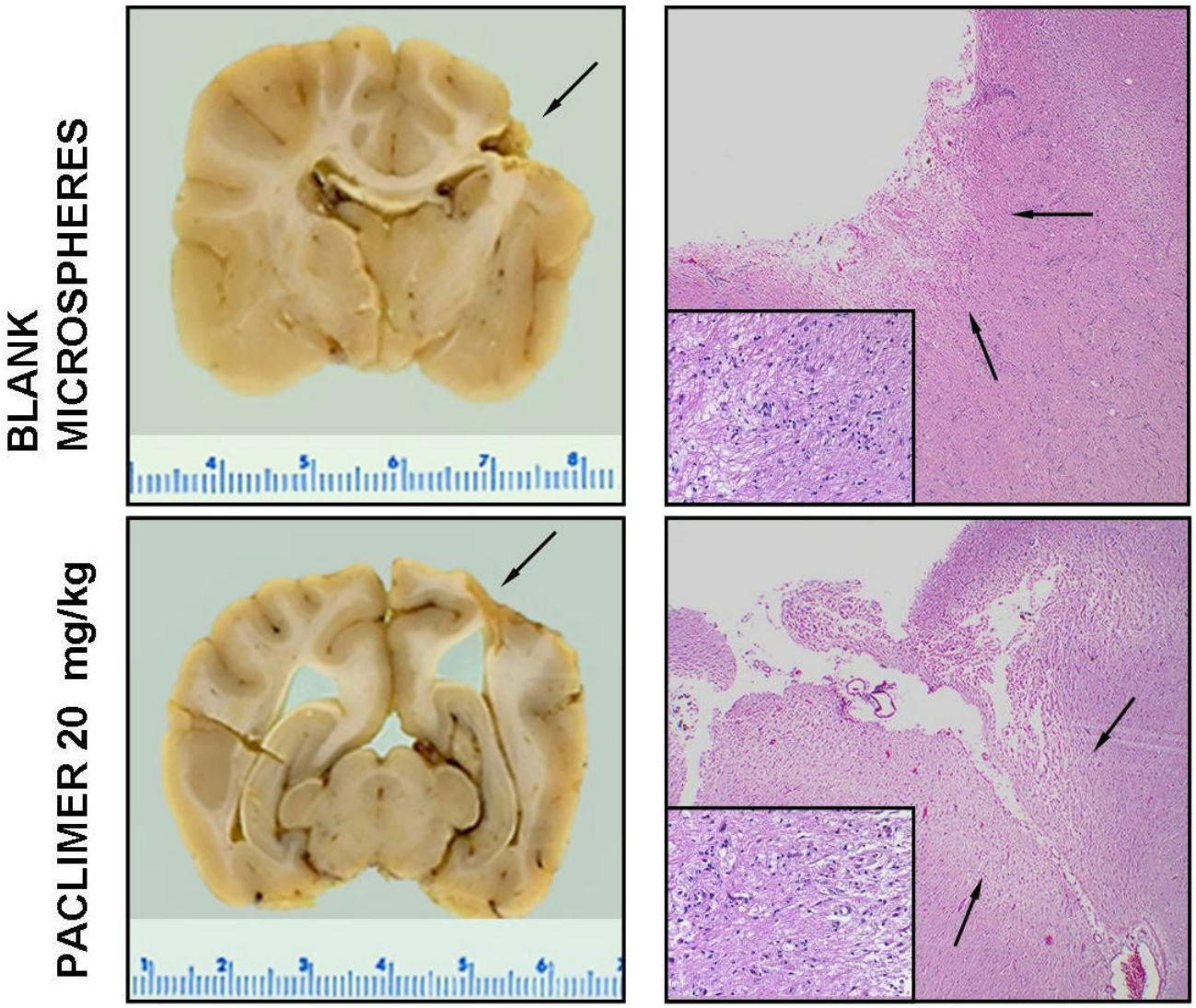

Figure 5.
Photographs of the brains of animal treated with: Paclimer® 20 mg/kg, Gross specimen (bottom left) and microphotograph of an H&E section (bottom right) and Blank microspheres, gross specimen (top left) and microphotograph of an H&E section (top right).

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Table 1

2 mg/kg Paclimer®				
Animal #	Treatment	Length of Survival (days post-implant)	Complications/Adverse Events	Outcome
1	Blank microspheres	120	None	No neurological deficit; scheduled sacrifice
2	Paclimer®	120	None	No neurological deficit; scheduled sacrifice
3	Paclimer®	123	Wound Infection	No neurological deficit; scheduled sacrifice
4	Paclimer®	123	Incisional Dehiscence	No neurological deficit; scheduled sacrifice
5	Paclimer®	123	Brain Abscess	No neurological deficit; scheduled sacrifice
6	Paclimer®	120	None	No neurological deficit; scheduled sacrifice
20 mg/kg Paclimer®				
Animal #	Treatment	Length of Survival (days post-implant)	Complications/ Adverse Events	Outcome
1	Blank microspheres	125	Wound Infection	No neurological deficit; scheduled sacrifice
2	Paclimer®	125	None	No neurological deficit; scheduled sacrifice
3	Paclimer®	62	Wound Infection	No neurological deficit; scheduled sacrifice
4	Paclimer®	101	Incisional Dehiscence	No neurological deficit; scheduled sacrifice
5	Paclimer®	59	Communicating Hydrocephalus	Lethargy; scheduled sacrifice
6	Paclimer®	99	None	No neurological deficit; scheduled sacrifice