Injectable Chemotherapeutic Microspheres and Glioma I: Enhanced Survival Following Implantation into the Cavity Wall of Debulked Tumors

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Purpose. Implantation of biodegradable polymers provides a powerful method to deliver high, sustained concentrations of chemotherapeutics to brain tumors. The present studies examined the ability of injectable polymeric microspheres, formulated to release carboplatin or BCNU for 2–3 weeks, to enhance survival in a rodent model of surgically-resected glioma.

Methods. Rat glioma (RG2) cells were implanted into the cortex of rats and allowed to grow for 10 days prior to surgical resection. Rats were given either surgical resection only, bolus injection (100 μg) or microspheres containing 10, 50, or 100 μg of carboplatin or BCNU. The microspheres were implanted, via hypodermic injection, either directly into the surgical cavity or into the tissue along the perimeter of the cavity.

Results. The order of survival among treatment groups was: no resection < resection only < bolus chemotherapy < sustained release chemotherapy. Carboplatin and BCNU did not differ in this respect and in each case, the enhanced survival achieved with sustained release was dose-related. However, the enhanced survival achieved with carboplatin was substantially greater when the microspheres were implanted into the perimeter wall of the resection cavity, compared to implantation into the cavity itself. The enhanced survival produced by carboplatin implants along the resection perimeter was associated with a significant attenuation of regrowth of the tumor. Finally, in a separate study in non-tumor brain, atomic absorption spectrophotometry revealed that while the microspheres produced significantly prolonged tissue levels of carboplatin relative to a bolus injection, carboplatin diffusion was limited to brain tissue extending primarily 0.5 mm from the injection site.

Conclusions. These data demonstrate: (1) that sustained delivery of chemotherapy is superior to equipotent bolus doses following tumor resection, and (2) that direct injection of sustained release microspheres into the tissue surrounding a growing tumor mass may provide superior effects over injections into the surgical cavity. They also suggest that successful implementation of this approach in humans may require measures or circumstances that improve upon the limited spatial drug diffusion from the implantation site.

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ABBREVIATIONS BCNU, 1, 3-bis[2-chloroethyl]-1-nitrourea; PCPP-SA, poly[bis(p-carboxyphemoxy)]propane-sebacic acid; PLG, poly (L-lactide) co-glycolide); RG2, rat glioma; PVA, polyvinyl acetate; PBS, phosphate-buffered saline; i.v., intravenous.

KEY WORDS: glioma; sustained release; microsphere; carboplatin; BCNU.

INTRODUCTION

Malignant gliomas continue to be resistant to treatment. Despite advances in surgery, radiation therapy, and continued improvements in systemic chemotherapeutic agents, the median survival of patients remains approximately one year from the time of diagnosis (1). Even when lifespan is increased, it is often associated with a loss in the quality of life. Numerous approaches over the past 10–20 years have focused on improving local exposure of brain tumors to chemotherapeutic drugs to enhance survival. One approach uses implantable, biodegradable polymers for local, sustained drug delivery directly to the tumor. Polymers containing chemotherapeutic agents have been extensively evaluated in animal models of brain tumors (2–6) and used most recently in the treatment of human glioma (7–9).

The most noteworthy effort to date uses poly[bis(p-carboxyphemoxy)]propane-sebacic acid copolymer (PCPP-SA) disks that release 1, 3-bis[2-chloroethyl]-1-nitrourea (BCNU) (7,8). FDA approval has been recently granted for their use as an adjunctive treatment to resection of glioma. Following surgical resection of tumors, the polymer disks are placed into the resulting cavity where the BCNU is released to diffuse into the surrounding tissue and residual tumor mass. The rationale of this approach is to prevent the preferential recurrence of glioma from the brain tissue near the resection cavity. While statistically significant patient benefit has been observed, controlled trials demonstrated that recurrence of the tumor is not prevented, but rather modestly delayed (e.g., median survival increased from 23 to 31 weeks) (8). One likely explanation for the relatively rapid recurrence of the tumor is that limited diffusion of BCNU leaves regions of local tumor infiltration with insufficient BCNU levels. If higher concentrations could be delivered directly to the area where tumor recurrence is most likely (i.e., the tissue within several cm of the surgical cavity) (10) then even greater efficacy might be achieved.

One way of increasing the likelihood that cytotoxic levels of a chemotherapeutic drug reach more distal regions of tumor infiltration would be to implant drug-loaded polymers directly into these sites. Unfortunately, it is impractical to implant monolithic devices such as polymer disks directly into tissue where tumor regrowth is most likely to occur. The size of the disks limits their use to situations where the tumor is surgically accessible and areas of known or likely tumor infiltration can only be targeted by diffusion of the drug from the cavity. An attractive alternative that would permit delivery directly to the site of residual tumor is the use of polymers such as poly (L-lactide) co-glycolide) (PLG) that are formed into small diameter microspheres (<100 um). Microspheres have been proven to be efficient systems for delivery of a wide range of chemotherapeutic drugs to the brain (11–13). They can be injected safely as a suspension allowing drug delivery to virtually any brain region with minimal invasiveness (14).

The following experiments are the first to evaluate the potential value of sustained release chemotherapy into the tissue surrounding a resection cavity. The experiments used a novel animal model of tumor resection intended to mimic the primary clinical application of this approach. Direct comparisons were

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made between sustained release of two different chemotherapeutic drugs, carboplatin and BCNU, within the cavity and into the perimeter, relative to equipotent bolus injections into both sites. The results provide compelling evidence for the superiority of peritumoral injections as a means of local chemotherapy. If applied to human glioma trials, and confirmed, this approach could positively impact the future use of polymeric delivery systems for treating brain tumors.

MATERIALS AND METHODS

Subjects

Male Fischer rats (N = 412; 200–220 g; Taconic Farms, Germantown, NY) were used in all studies. The rats were housed in pairs in polypropylene cages with free access to food and water. The vivarium was maintained on a 12 h light:12 h dark cycle with a room temperature of 22 ± 1°C and relative humidity level of 50 ± 5%. All studies were in compliance with the rules set forth in the Guide for the Care and Use of Laboratory Animals.

Tumor Cell Implantation

RG2 cells were maintained and prepared for implantation as previously described (15). Rats were anesthetized with an intramuscular injection of ketamine (33 mg/ml), xylazine (10 mg/ml) and acepromazine (1.6 mg/ml) and placed in a stereotaxic instrument. Using a 10 μl Hamilton syringe and a 22 gauge needle, cells were injected unilaterally into the cortex (5×10^4 cells/2 μl) at the following coordinates; A-P (−1.0 mm), L (+4.0 mm) and V (2.5 mm) (16).

Total Entrapment and *In Vitro* Release from Microspheres

Microspheres (PLG, Microsorb 50/50 DL, MW = 10 kD, Alkermes Inc., Wilmington, Ohio) were fabricated to provide a carboplatin loading density of 10% (w/w) and a BCNU loading density of 15% (w/w). Carboplatin-loaded (Sigma Chemical) microspheres were fabricated using a coacervation process. Initially, 1.0 gram of PLG was dissolved in 10 mls of methylene chloride. 110 mg of ground/sieved carboplatin (<5 μm diameter) was added to this solution, and sonicated for 5 minutes followed by vigorous vortexing. Nine mls of poly (dimethylsiloxane) (Aldrich, 350 cst) was then added and the resulting emulsion was mixed and stirred with 1 liter of heptane for 2 hours. The microspheres were collected by filtration and the solvent allowed to evaporate. The microspheres were washed twice with 0.8% triton X-100, suspended in a 1% polyvinyl acetate (PVA) solution and sieved through 70 and 40 μM cell strainers. BCNU-loaded (Sigma Chemical) microspheres were fabricated by a solvent evaporation process. One gram of PLG and 110 mg of BCNU were dissolved in 7.5 mls of methylene chloride. The solution was added to a 100 ml 4-neck glass reaction flask containing 40 mls of 0.75% PVA. The organic phase was dispersed in aqueous medium and maintained at room temperature for 10 minutes prior to being gradually raised to 40°C over 50 minutes. The solution was then maintained at 40°C for an additional 50 minutes before cooling to room temperature and collecting the microspheres by filtration. All microspheres were washed with distilled water prior to being frozen at −70°C and lyophilized under 50 μTorr for 2 days. Blank (non-loaded) microspheres were treated in an identical manner except that carboplatin or BCNU was omitted from the procedure.

In vitro release of carboplatin and BCNU was determined by incubating the microspheres in phosphate buffered saline (PBS) at 37°C. At 1 hour, 8 hours, 1, 3, 7, 14, and 21 days (n = 3/time point), the solution was removed and the amount of carboplatin or BCNU released was measured. The total amount of drug loaded was determined by dissolving 1 mg of the microspheres in 1 ml of methylene chloride. Carboplatin samples were analyzed using graphite furnace atomic absorption spectrophotometry equipped with Zeeman background correction. Platinum levels were determined by comparing the signal of the sample against known platinum calibration standards at a wavelength of 265.9 mm. BCNU was measured spectrophotometrically using the assay of Bratton and Marshall (17) as modified by Loo and Dion (18).

Residence Time and Distribution of Carboplatin in Normal Brain

To determine the residence time and distribution of carboplatin *in vivo*, non-tumor bearing animals (N = 42) received implants of microspheres into the striatum at the following coordinates: A-P (+2.0 mm), L (+3.0 mm) and V (−6.5 mm) (16,19). For implantation, the microspheres were suspended (10% PLG w/v) in a solution of 0.9% saline, 0.1% Tween and 3.0% carboxymethylcellulose (low viscosity). Microspheres (1 mg/10 μl) containing a total of 100 μg carboplatin were injected at a rate of 2 μl/minute using a 10 μl Hamilton syringe with an attached 23 gauge needle. Separate sets of animals received a single bolus injection of 100 μg of carboplatin into the same site. At 1 minute, 1 hour, and 1, 3, 7, 14, and 21 days later (n = 3/time point), the animals were euthanized via CO_2 asphyxiation and the brains rapidly removed. The implanted striatum was blocked at the dorsal aspect of the corpus callosum and the brain tissue surrounding the needle tract was dissected into discrete regions using 2 stainless steel microdissection needles. The first section extended from the needle tract outward for 0.5 mm and the second extended from 0.5 mm to 1.5 mm from the needle tract. Tissue samples were digested and incubated in 0.5 mls of Soluene at 37°C for 8 hours. 0.5 mls of methylene chloride was then added to dissolve the microspheres and platinum levels were measured as described above.

Tumor Resection and Microsphere Implantation

Ten days following implantation of RG2 cells, anesthetized animals were placed into a stereotaxic device and a craniotomy was made extending approximately 2 mm radially from the original burr hole. The dura was excised and using a hand-held aspirative device, gross resection of the tumor from the cavity was performed leaving behind a small margin-positive resection area to evaluate the effects of the drug-loaded microspheres on regrowth of the tumor and survival of the animals. Tumor volumes, the volume of the cavity resulting from the resection procedure and the amount of residual tumor mass left following the resection were determined in separate animals at day 10 (N = 6/group).

Three hundred and one animals were used in survival

studies to evaluate the effects of the drug-loaded microspheres when implanted either directly into the resection cavity or into the brain tissue along the perimeter of the cavity. For implantation directly into the cavity animals received PLG (10 μl of PLG-containing suspension) containing a total of 10 μg, 50 μg, or 100 μg carboplatin or BCNU. Identical amounts of microspheres were implanted in all cases by adding blank microspheres to the suspension. A separate set of animals received a one-time bolus injection of 100 μg of either carboplatin or BCNU directly into the resection cavity. Additional animals received either no treatment or surgical resection of the tumor only.

For implantation into the tissue along the perimeter of the resection cavity, separate groups of animals received the same total amount of microspheres that were implanted directly into the resection cavity, except that it was equally divided into 4 separate 2.5 μl aliquots. Tissue injections of PLG containing 2.5 μg (10 μg total), 12.5 μg (50 μg total), or 25 μg (100 μg total) carboplatin or BCNU into the 4 sites were made approximately 0.5 mm from the edge of the resection cavity and in a diamond-shaped configuration with one site rostral and one caudal to the cavity and the other two sites lateral to the cavity. This procedure resulted in 4 implant sites approximately 2 mm apart from each other. Separate animals received a carboplatin or BCNU bolus (25 μg/site; 100 μg total). Animals were monitored daily and any animal showing signs of morbidity was euthanized and that date recorded for calculating survival data.

Effect of Carboplatin-Loaded Microspheres on Tumor Regression

A separate set of animals (N = 36) was used to determine the effects of sustained release of carboplatin on tumor regrowth following surgical resection. Animals received intracortical implants of RG2 cells as described above. Ten days later, animals were divided into 3 treatment groups (N = 12/group). In the first, animals received a surgical resection only and were sacrificed 28 days following tumor implant. This time point was chosen from the previous survival studies and represented a point at which animals were beginning to die but left a sample size large enough for histological evaluation. In the second group, animals received a resection plus carboplatin-loaded microspheres injected into the surrounding tissue (25 μg/site) and were also sacrificed 28 days following tumor implant. This group permitted direct comparisons with the untreated animals killed at the same time point. The third group of animals received a resection plus 4 carboplatin-loaded microsphere injections into the surrounding tissue (25 μg/site) and were sacrificed 38 days following tumor implant. This time point was chosen post-hoc since significant numbers of animals were dying but the remaining sample size was large enough to permit an analysis of the tumor volumes in the PLG-treated remaining animals. Anesthetized animals were transcardially perfused using 200 mls of ice-cold 0.9% saline followed by 500 mls of Zamboni's fixative. Sections were cut at 30 μM intervals on a cryostat and stained for hematoxylin and eosin (H&E). Tumor volumes in all animals were calculated using the following standard trapezoidal equation:

$$\frac{\text{Sum of areas } (\text{mm}^2)}{\text{Number of Sections}} \times \text{Extent of Tumor}$$

Statistical Analysis

Survival after each treatment was analyzed using Kaplan-Meier survival curves. Non-parametric Kruskal-Wallis statistics were used to determine overall treatment effects using the day of death as the nonparametric variable (JMP, SAS Institute Inc., Cary, N.C.). The nonparametric modification of the Neuman-Keuls test was used for subsequent pair-wise comparisons. The effects of carboplatin-loaded microsphere injections on tumor volume were compared using a one-way analysis of variance. Statistical significance in all cases was defined as $p < 0.05$.

RESULTS

In Vitro Release of Carboplatin and BCNU From Microspheres

The amount of carboplatin entrapped within the microspheres matched the predicted 10% loading density while the absolute amount of BCNU entrapped was slightly less than predicted (12.7% vs the predicted 15%). Release of both compounds during the first 24 hours was similar with approximately 13% of carboplatin and 10% of BCNU released. Carboplatin release was sustained until 100% of the drug was released between 14 and 21 days. BCNU release was similar with the exception that a larger burst of release occurred between day 1 and day 3. While only 30% of carboplatin was released within 3 days, 60% of BCNU was released over the same 3 day period. Total release of BCNU was more rapid, with 100% release occurring by 14 days (Fig. 1).

Residence Time and Distribution of Carboplatin in Normal Brain

Delivery of carboplatin via the PLG microspheres significantly prolonged the time that tissue levels of carboplatin were increased in brain tissue when compared to a single bolus injection. Carboplatin tissue levels in normal brain decreased 76% by 1 day and >99% by 3 days following a bolus injection, relative to the earliest time point examined (1 minute). In contrast, carboplatin levels decreased only 23% after 1 day and 44% after 3 days following implantation of microspheres. Carboplatin levels remained elevated in brain tissue relative to bolus administration until 14 days, at which time they had decreased 95%. The diffusion of carboplatin from the microspheres was limited to a region of brain that extended about 0.5 mm from the injection site. As shown in Fig. 2, levels of carboplatin were 80–90% lower in tissue 0.5–1.5 mm from the injection site compared to the region within 0.5 mm of that site during the first 24 hours. Carboplatin was not detected beyond 0.5 mm from the injection site after 1 day.

Survival Following Implantation of Carboplatin-Loaded Microspheres

Ten days following implantation, the cortical tumor had grown to an average volume of 8.06 ± 1.55 mm^3. The tumor

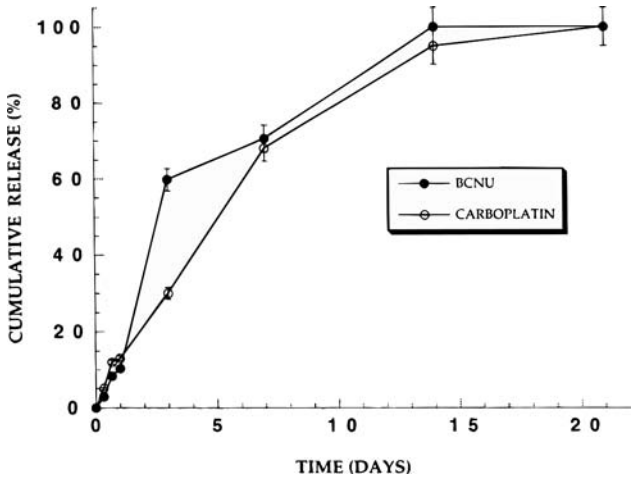

Fig. 1. In vitro carboplatin and BCNU release from PLG microspheres. Microspheres were incubated in PBS at 37°C and the amount of platinum released from the microspheres was determined using atomic absorption spectrophotometry while the amount of BCNU released was measured using spectrophotometry.

extended approximately 1 mm dorsal to the cortical surface and ventrally to the corpus callosum. Surgical resection of the tumor eliminated most of the tumor mass and resulted in a cavity with a mean volume of 6.92 ± 0.74 mm^3 (see Fig. 6). Residual tumor volume was 0.94 ± 0.24 mm^3 or 8.8% of the original tumor volume.

The cortical RG2 tumor was uniformly fatal to all nontreated animals, with a median survival of 20 days and maximum survival of 22 days post-tumor implantation (Fig. 3). Surgical resection alone significantly increased ($p < 0.01$) median survival by 10 days (50%) relative to untreated animals, with all animals dying by 36 days post-implantation (increase of 64%). Sustained release of 10 and 50 μg of carboplatin injected directly into the cavity did not impact survival relative to surgery alone ($p > 0.05$). The higher, 100 μg dose modestly increased survival ($p < 0.01$), resulting in a median survival of 36 days (increase of 20% relative to surgery alone) and a maximum survival of 49 days (increase of 36%). A bolus injection of carboplatin (100 μg) increased median survival by 20% ($p < 0.01$) which was not significantly different ($p > 0.1$) from survival following 100 μg of sustained release carboplatin (Fig. 3).

Injection of microspheres directly into the tissue along the perimeter of the resection cavity enhanced survival more than injections into the cavity (Fig. 4). The effect was dose-related, with the lowest dose of carboplatin (10 μg) having no effect, 50 μg increasing median survival 57% and maximum survival 100% and 100 μg of carboplatin increasing median survival by 110% and maximum survival by 400%. Two animals receiving the 100 μg dose of sustained release carboplatin into the cavity wall survived the duration of the experiment (180 days post tumor-implantation). Histological analysis of H&E-stained sections from these animals revealed that the tumors had been completely eradicated by sustained release of carboplatin. A bolus of carboplatin (100 μg) into the surrounding tissue (100 μg) improved median survival by 17% ($p < 0.01$), though this effect was much less than that achieved with sustained release ($p < 0.0001$).

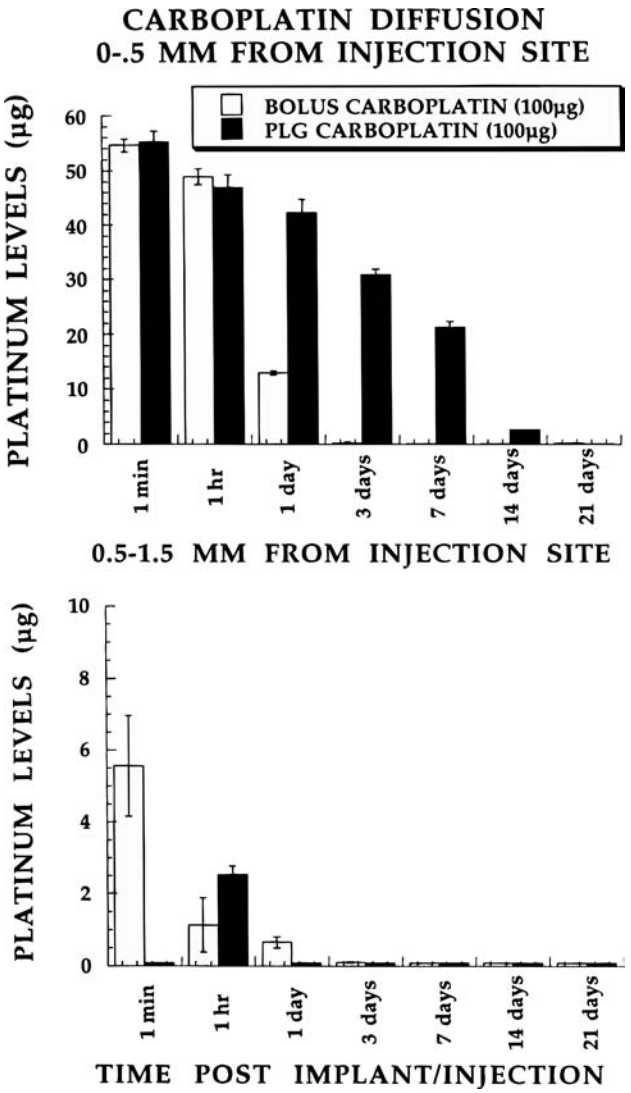

Fig. 2. In vivo characterization of carboplatin diffusion from microspheres. Animals received intrastriatal implants of microspheres containing 100 μg of carboplatin or a single bolus injection of the same amount (100 μg) of carboplatin. At various times following injection the tissue surrounding the injection site was dissected into two regions: one comprising the tissue from the center of the site outward 0.5 mm (top) and a second extending from 0.5 to 1.5 mm (bottom) outward from the injection tract. Tissue levels of platinum were determined using atomic absorption spectrophotometry. Note that following injection of both the microspheres or the bolus of carboplatin, the diffusion of carboplatin was limited primarily to the tissue within 0.5 mm of the injection tract. Note also that tissue levels of carboplatin decreased rapidly within the first 24 hours of the bolus injection but remained elevated for 7–14 days following implantation of the sustained release microspheres.

Effect of Carboplatin-Loaded Microspheres on Tumor Volume

A separate study assessed the relationship between the enhanced survival produced by sustained release of carboplatin and changes in tumor volume (Figs. 5 and 6). Animals received the same tumor resection plus four implants of carboplatin-loaded microspheres into the perimeter of the cavity (100 μg

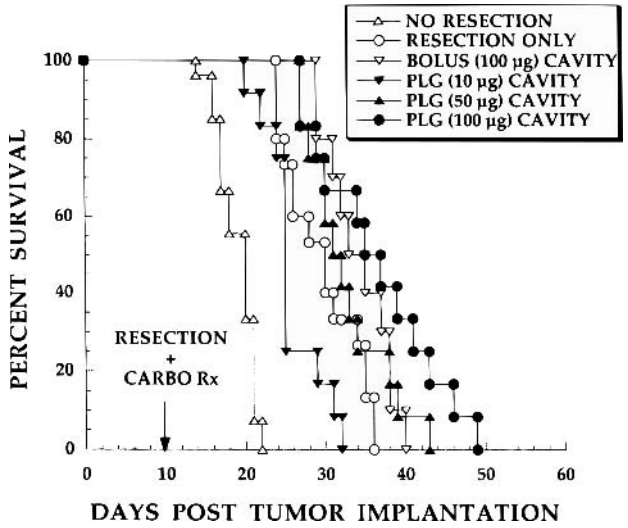

Fig. 3. Survival following a bolus injection of carboplatin (100 μg) or implantation of carboplatin-loaded microspheres (10 μg, 50 μg, or 100 μg total) directly into a tumor resection cavity. Surgical resection of the tumor alone produced a modest increase in survival relative to animals receiving no resection. While a bolus injection of carboplatin did not impact survival, sustained release of carboplatin produced significant dose-related increases in survival relative to resection only.

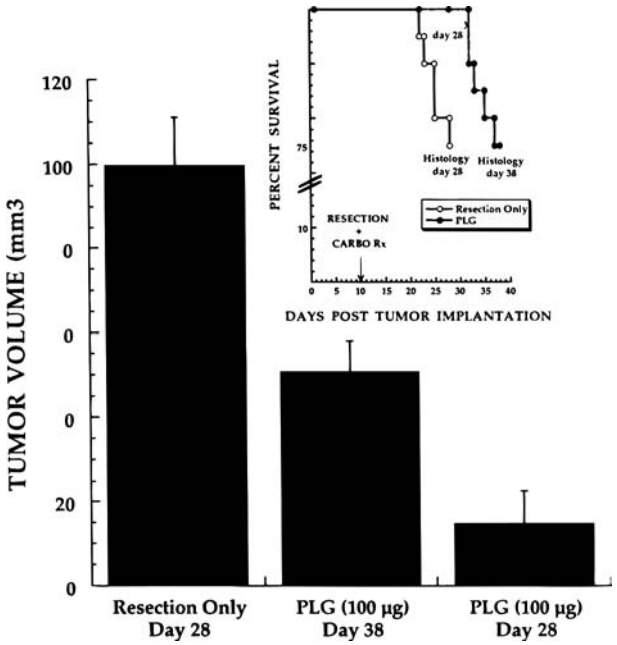

Fig. 5. Survival curves and tumor volumes for animals receiving either a surgical resection only, surgical resection plus 100 μg sustained release carboplatin and sacrificed 28 days following tumor implantation, or surgical resection plus 100 μg sustained release carboplatin and sacrificed 38 days following tumor implantation. The enhanced survival produced by implantation of carboplatin-loaded microspheres was paralleled by a significant reduction in the regrowth of the previously resected tumor.

total), as in the survival studies. These animals were compared to those receiving tumor resection only and sacrificed 28 days following tumor implant (a time point where a small but reliable proportion of resected-only animals had begun to die). As in the prior survival study, implantation of microspheres releasing carboplatin into the tissue along the perimeter of the cavity enhanced survival. At day 28, none of the carboplatin microsphere-implanted animals died, compared to 25% of the resection-only animals. An analysis of tumor volume revealed that at this time point, the tumor volumes were significantly less (Fig. 6C) in the animals treated with sustained release of carboplatin, compared to animals receiving resection only (Fig. 6B) (14.8 mm^3 vs 99.5 mm^3). We next evaluated the residual tumor of the resection plus carboplatin microspheres animals at day 38 (because at that time point the 75% survival rate was identical to the survival at day 28 for the resection-only animals). Despite similar survival proportions at this point in time, the tumors in the resection plus PLG-carboplatin animals days were significantly smaller (Fig. 6D), relative to the resection only group at 28 days, (50.7 mm^3 vs 99.5 mm^3).

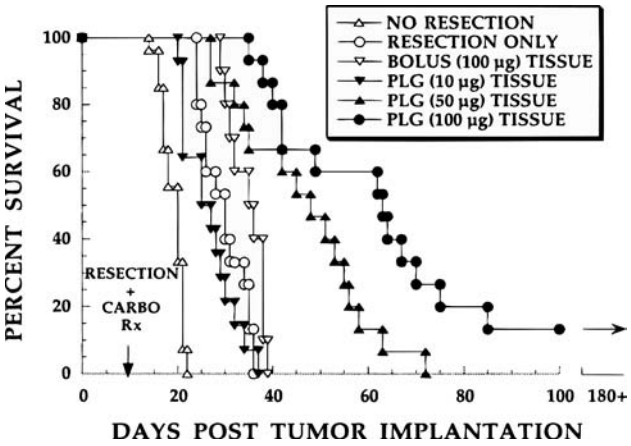

Fig. 4. Survival following a bolus injection of carboplatin (25 μg/site) or implantation of carboplatin-loaded microspheres (2.5 μg, 12.5 μg, or 25 μg/site) into 4 sites within the tissue surrounding a tumor resection cavity. The same total amount of carboplatin injected into the cavity was equally dispersed over 4 implant sites (i.e., 25 μg/site for a total of 100 μg). A bolus injection of carboplatin modestly increased survival, while sustained release of both 50 μg and 100 μg of carboplatin produced more robust, dose-related increases in survival relative to resection only. The intermediate and highest doses of sustained release carboplatin into the perimeter of the tumor produced superior effects to that obtained with sustained release directly into the resection cavity. Two animals (out of 12) survived the duration of the experiment (180 days).

Survival Following Implantation of BCNU-Loaded Microspheres

As in the prior survival study, surgical resection of the cortical tumor significantly increased median (50%) and maximum survival (95%) relative to untreated animals ($p < 0.01$) (Fig. 7). Direct injections of BCNU-containing microspheres into the resection cavity increased survival in a dose-dependent manner. Sustained release of 10 μg of BCNU did not impact survival relative to surgery alone ($p > 0.05$), while 50 μg of BCNU enhanced median and maximum survival (14% and 28%, respectively) and 100 μg of BCNU increased median survival 36% and maximum survival 56% (p's < 0.01). A bolus

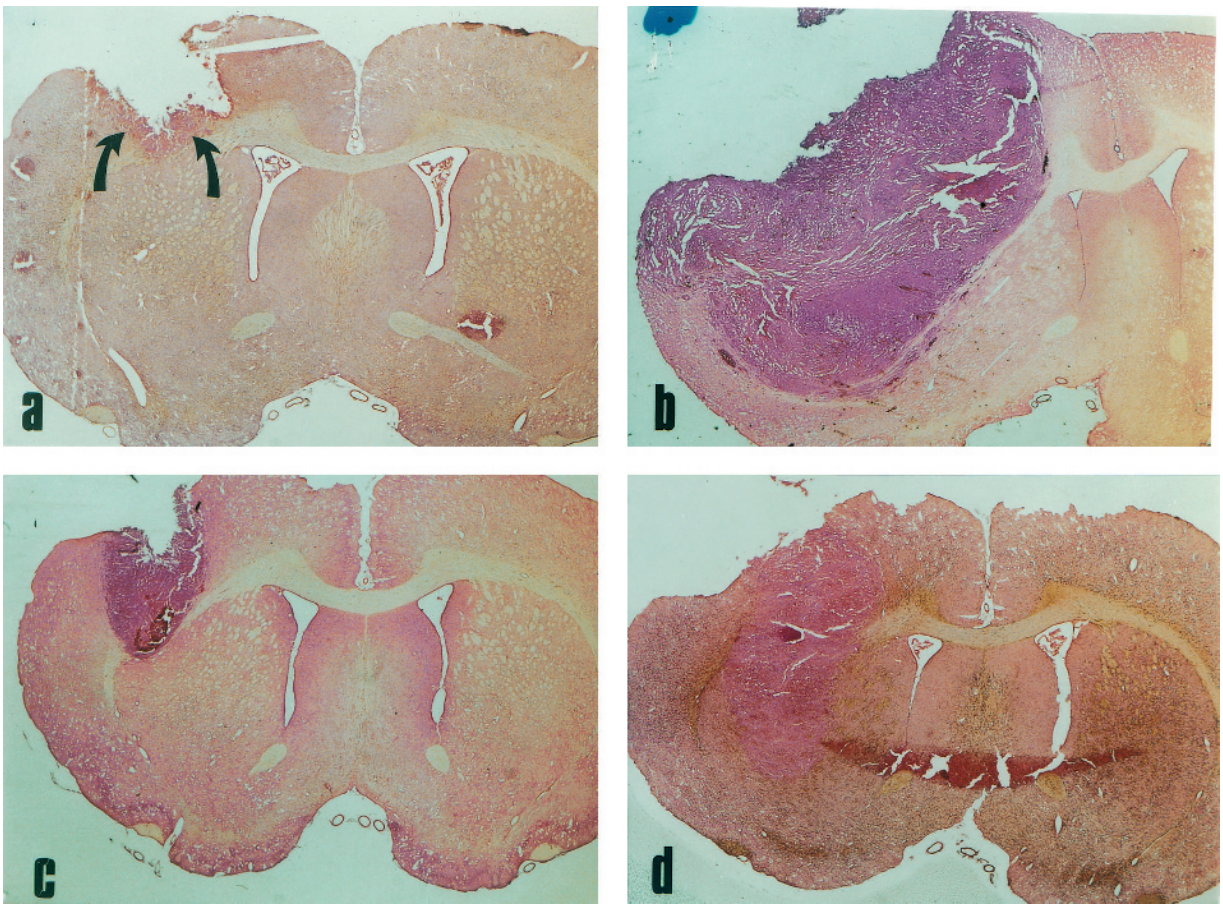

Fig. 6. Photomicrographs of H&E-stained brain tissue following resection of cortical tumor and implantation of carboplatin-loaded microspheres. (A) Example of cavity resulting from resection of RG2 cells 10 days following implantation into the cortex. The solid arrows illustrate residual tumor cells along the wall of the cavity. The remaining panels show representative sections from animals in Figure 5. (B) Section of tumor from an animal that received a tumor resection only and was sacrificed 18 days later (28 days following tumor implantation). Note the extensive regrowth of the tumor that encompasses the majority of the hemisphere. (C) Tissue section containing a tumor from an animal that received a tumor resection plus carboplatin-loaded microspheres implanted into the tissue along the perimeter of the cavity. This animal was also sacrificed 18 days later (28 days following tumor implantation). Note that implantation of microspheres has significantly slowed the regrowth of the tumor which now occupies a portion of the surgical cavity. (D) Section from an animal receiving the same treatment as in panel C but sacrificed 38 days following tumor implantation. Note that while the tumor has regrown to a greater extent, its growth has still been significantly inhibited by the implantation of carboplatin-containing microspheres.

injection of BCNU (100 mg) did not increase survival (p . 0.1). Sustained release of BCNU was superior to a bolus injection of BCNU (p , 0.05).

Sustained release of BCNU into the perimeter of the cavity also produced a dose-dependent increase in survival (Fig. 8). While the lowest dose (10 mg) of BCNU did not impact survival (p . 0.1), sustained release of 50 mg and 100 mg of BCNU significantly increased survival relative to surgery alone (p , 0.001). Implantation of 50 mg increased median survival 32% and maximum survival 59%, while 100 mg increased median survival by 61% and maximum survival by 72%. A bolus injection of BCNU into the surrounding tissue (100 mg) improved median and maximum survival by 25% and 39%, respectively (p's , 0.01). Sustained release of BCNU (100 mg) was superior to a bolus injection of BCNU (p , 0.01). While injections of BCNU into the tumor perimeter produced consistently greater survival than equivalent injections into the cavity, these differences did not satisfy conventional statistical levels of significance (p 5 .10). Nonetheless, survival following 50 mg of sustained release BCNU injected into the perimeter was nearly identical to survival following 100 mg of BCNU injected into the cavity.

DISCUSSION

These studies used a novel model of surgically resected glioma to establish several fundamental points regarding the use of sustained release microspheres as a treatment for brain tumors. Collectively they establish that: (1) survival in a clinically-relevant glioma model can be enhanced by peritumoral injection of chemotherapeutic microspheres and it occurs because of a significant reduction in tumor burden, (2) microspheres provide a convenient and easily injectable system for sustained release of chemotherapeutic drugs into both the cavity produced by surgical resection as well as the tissue along the perimeter of that cavity, (3) sustained delivery of chemotherapy is superior to equipotent bolus doses, (4) direct injections of sustained release microspheres into the tissue surrounding a

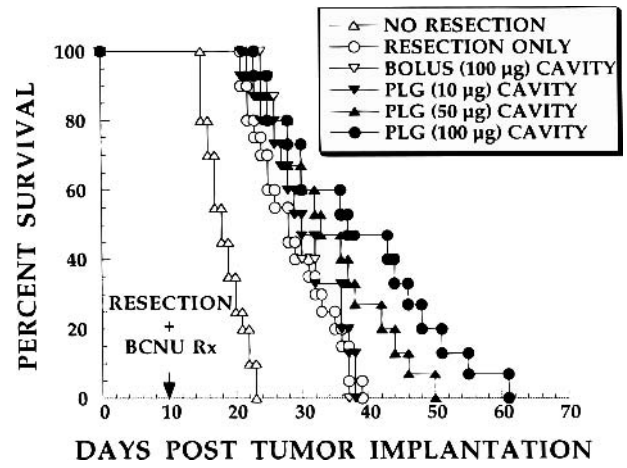

Fig. 7. Survival following a bolus injection of BCNU (100 μg) or implantation of BCNU-loaded microspheres (10 μg, 50 μg, or 100 μg total) directly into a tumor resection cavity. Surgical resection of the tumor alone produced a modest increase in survival relative to animals receiving no resection. While a bolus injection of carboplatin did not impact survival, sustained release of BCNU produced significant dose-related increases in survival relative to resection only.

growing tumor mass may provide superior effects over injections into the surgical cavity, and (5) the use of multiple implants of microspheres might be required to improve upon the limited spatial drug diffusion from the injection site.

That sustained release is superior to bolus injections, even when the bolus is equivalent to the total amount of drug released from the microspheres, is an important and often ignored concept. In the present studies, tissue levels of carboplatin remained elevated following sustained release but not bolus injections of carboplatin. Within 3 days of a bolus injection, carboplatin levels were decreased 99%, while sustained release resulted in tissue levels that decreased only 44% after 3 days and did not decline to <1% until 14 days after implantation. Independent of whether the microsphere injections were made directly into the surgical cavity or into the tissue along the perimeter of the cavity, sustained release was superior to the burst of chemotherapeutic drug delivered to the tumor following a bolus injection. The retention of BCNU was not measured in the current experiments but is likely shorter than that of carboplatin, given its short plasma half-life (<15 minutes vs 2–3 hours for carboplatin), and rapid clearance from the extracellular space (20). The superiority of sustained release BCNU is similar to the results of Buahin and Brem (21) who found that sustained release, but not direct intratumoral bolus injections of BCNU, enhanced survival in glioma-bearing rats.

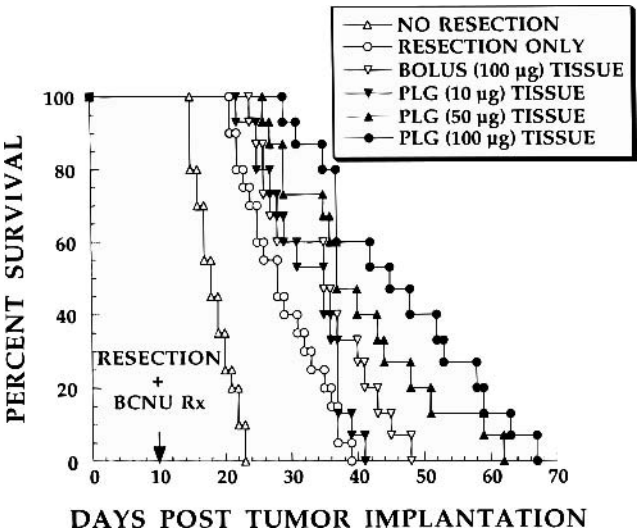

Fig. 8. Survival following a bolus injection of BCNU (25 μg/site) or implantation of BCNU-loaded microspheres (2.5 μg, 12.5 μg, or 25 μg/site) into 4 sites within the tissue surrounding a tumor resection cavity. The same total amount of BCNU injected into the cavity was equally dispersed over 4 implant sites (i.e., 25 μg/site for a total of 100 μg). A bolus injection of BCNU modestly increased survival, while sustained release of both 50 μg and 100 μg of BCNU produced more robust, dose-related increases in survival relative to resection only. Note also, that no advantage was seen for delivery of BCNU when implanted into the tissue surrounding the resection cavity vs directly in the cavity.

These studies provide important new information regarding the potential superiority of sustained release chemotherapeutic delivered to the tissue along the perimeter of a resection cavity vs delivery directly into the cavity. While surgical resection of glioma removes the majority of the tumor mass and increases the survival of patients, recurrence of the tumor typically occurs within centimeters of the original tumor mass because remaining cells within the surrounding tissue are not detectable, or cannot be removed during the neurosurgical debulking procedure (10). Injecting microspheres into the tissue along the perimeter of a resection cavity increases the likelihood of achieving cytotoxic drug concentrations in these regions, perhaps further increasing survival. The present experiments are the first to directly test this possibility and provide fundamental information regarding the feasibility and efficacy of direct peritumoral injections of chemotherapeutic drug loaded polymeric carriers. Microspheres dispersed over 4 implant sites along the perimeter of cavity, produced markedly greater survival, compared to the same dose of microspheres injected directly into the tumor cavity. This effect was most obvious with carboplatin, where enhanced survival was robust and dose-related. The highest dose of sustained release carboplatin into the perimeter of the cavity increased median survival by 75% and maximum survival by 267% over that with implantation directly into the cavity. The effects achieved with BCNU in the tissue surrounding the cavity were less robust than those with carboplatin. Although the survival achieved with BCNU microspheres implanted into the cavity wall was not statistically greater than that achieved with injections directly into the cavity, consistently greater survival was still seen with injections into the perimeter of the cavity. In fact, 50 μg of sustained release BCNU into the perimeter was at least as potent as 100 μg of sustained release BCNU into the cavity. While BCNU may benefit less from peritumoral implantation than does carboplatin, it also seems likely that at least some of the difference in survival reflect differences in the release profile of the two agents. The in vitro release of BCNU was much more rapid during the first three days, with approximately 30% more BCNU than carboplatin being released. The lower levels of BCNU remaining in the microspheres beyond 3 days may have allowed the tumor to return more quickly, relative to the more extended carboplatin release time.

Several factors may have accounted for the differences in the release profiles of carboplatin and BCNU including the encapsulation technique (co-acervation for carboplatin and solvent precipitation for BCNU), the relative hydrophilicity of the two compounds, the molecular weight differences between carboplatin and BCNU, the polydispersity of the size of the microspheres, and the distribution of the drug within the microspheres. Each of these factors, alone or in combination, could contribute significantly to the release of drugs from the polymeric micropsheres. Future studies examining the relationship between the duration of release, dose delivered, and cell killing in vivo should consider each of these factors when optimizing sustained delivery of chemotherapeutics for treating brain tumors.

If the superiority of sustained release into the peritumoral tissue is verified in human glioma patients, the design of future studies using polymer delivery systems could be dramatically changed. Recently, Menei and colleagues (13) reported the results of a preliminary uncontrolled clinical study in which 5-FU-loaded microspheres were injected into the cavity wall produced by surgical resection of glioma in 8 patients. The procedure was well tolerated and at the time of the report, median survival was 98 weeks with 1 patient in remission at 153 weeks post-treatment. These impressive results suggest that polymeric delivery of chemotherapeutic drugs directly into the cavity wall may provide a practical and more effective means of treating glioma over that normally expected with delivery into the cavity itself. Future studies will require larger cohorts and direct comparisons between groups receiving systemic therapy as well as sustained release directly into the surgical cavity before these preliminary data can be safely interpreted.

Diffusion of chemotherapeutic drugs from sustained release polymers into the surrounding brain tissue is generally limited (22–24). In the present studies, we confirmed that carboplatins diffusion in non-tumor brain is no more than 0.5 mm from the injection site. The short distribution distance and steep concentration gradients achieved by diffusion could severely limit diffusion-based delivery to tumors and other intraparenchymal diseases (22). However, drug distribution in the brain is not only controlled by diffusion, for an organized flow of fluids also exists within the brain (25). Tissue pressure gradients push extracellular fluids by bulk flow within the extracellular spaces along white matter tracts and within expanded pericapillary and perivascular spaces (26,27). This system provides the opportunity to distribute compounds to portions of the brain normally unreachable by simple diffusion alone. These paths of least resistence may also help to explain the inability to cure brain tumor patients despite the use of aggressive local therapies. Fluid channels, associated with these pathways, provide an avenue for tumor cells to migrate, enabling the dissemination of tumors outside the conventional surgical or radiotherapy field. Indeed, clinical studies confirm that white matter tracts are a common site of tumor recurrence (28–30). Normally, the limited penetration of drugs into brain tumors would necessitate the use of multiple implants to sculpt a drug field encompassing the entire tumoral and peritumoral region. However, the presence of regions of higher bulk flow provides the opportunity to anticipate the most likely areas of tumor recurrence, with specific implications for the continued refinement of local sustained release chemotherapies. Implantation of drug-loaded microspheres within the perimeter of the resection cavity might permit higher concentrations of drug to travel along these paths where tumor cells are lodged. Alternatively, sustained release polymeric microcarriers could be implanted directly within those regions of likely tumor cell migration. The use of injectable microspheres as part of the chemotherapeutic armatorium would permit chemotherapy to encompass the primary tumor site and its most likely route of infiltration.

The data from these studies detail several new and compelling findings regarding the use of sustained release chemotherapy for treating glioma. Using an acute animal model of surgically resected glioma, the superiority of sustained release over bolus drug injections was confirmed. Furthermore, the results provide the first direct evidence that injections of sustained release microspheres into the tissue surrounding a growing tumor mass may provide superior effects over injections into the surgical cavity. Given the typical constrained drug diffusion within the brain, the use of injectable microspheres implanted into multiple peritumoral sites might provide one means of improving the limited spatial drug diffusion from the implantation site. Further work is required to establish the generality of the effects observed, especially in human gliomas. If confirmed in human glioma studies, those data would suggest that patients might benefit significantly from injection of microspheres that provide a localized and sustained delivery of chemotherapeutic drugs directly to regions where tumor recurrence is most likely.

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