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

Dwaine F. Emerich,1,3 S. R. Winn,2 Yunhua Hu,2 Joanne Marsh,1 Pamela Snodgrass,1 Denise LaFreniere,1 Tania Wiens,1 Brant P. Hasler,1 and Raymond T. Bartus1

Received February 14, 2000; accepted April 11, 2000

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.

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-carboxyphenoxy)]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 µm). 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
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 × 10^5 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 released 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 CO2 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.