Injectable Chemotherapeutic Microspheres and Glioma II: Enhanced Survival Following Implantation into Deep Inoperable Tumors

Dwayne F. Emerich,1,3 S. R. Winn,2 Pamela Snodgrass,1 Denise LaFreniere,1 Mary Agostino,1 Tania Wiens,1 Hua Xiong,1 and Raymond T. Bartus1

Purpose. Delivery of chemotherapeutics using implantable, biodegradable polymers provides a potentially powerful method of treating brain tumors. The present studies examined the ability of injectable microspheres, formulated to release carboplatin or BCNU for 2–3 weeks, to enhance survival in a rodent model of deep, inoperable glioma.

Methods. Rat glioma (RG2) cells were implanted into the striatum of rats. In a first experiment, the tumors were allowed to grow for 3 days, followed by either no treatment, bolus chemotherapy (100 μg), or implantation of microspheres containing 10, 50, or 100 μg of car- boplatin. The microspheres were injected, via hypodermic injection, directly into the center of the small, 3-day-old tumors. In a second experiment, tumors grew for 8 days prior to treatment with either carboplatin- or BCNU-loaded microspheres. The microspheres were then injected either directly into the center of these larger tumors or into three sites along the perimeter of the tumor. Separate sets of animals received bolus chemotherapy (100 μg) into either the tumor center or around the tumor perimeter.

Results. Injection of carboplatin-loaded microspheres into the center of the small 3 day old, tumors produced dose-related increases in survival. When injections of carboplatin- or BCNU-loaded microspheres were made into the center of the larger, 8-day-old tumors, survival was not enhanced. However, when the microspheres were injected into the larger tumors, sustained-release chemotherapy did significantly prolong survival. Bolus chemotherapy was less effective than sustained release chemotherapy.

Conclusions. Together, these data: (1) demonstrate that sustained delivery of chemotherapy in or near the tumor site is superior to equipotent bolus doses in inoperable tumors, (2) demonstrate that injection of sustained release microspheres into the tissue surrounding a growing tumor may provide superior effects over injections directly into the tumor mass, and (3) suggest that this approach may provide a useful means of selectively delivering chemotherapeutics to tumors or portions of tumors that cannot otherwise be treated with conventional surgical approaches.

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

INTRODUCTION

Despite aggressive treatment regimens, the median survival of patients remains approximately one year from the time of diagnosis and cases of long-term disease-free survival in adults are rare (1). A significant limiting factor in treating glioma is the inability to deliver therapeutic concentrations of chemotherapeutic drugs to the tumor without incurring unacceptable systemic side effects. Developing approaches to increase local exposure of brain tumors to chemotherapeutic drugs, without increasing systemic toxicity, would be a valuable means of optimizing the antitumor activity of currently used chemotherapeutic drugs.

Implantable, biodegradable polymers provide a useful and practical means of maximizing the efficacy of antineoplastic drugs by providing vehicles for local and sustained drug delivery directly to the tumor. Polymeric carriers for chemotherapeutic agents have been extensively evaluated in animal models of brain tumors (2–6) and most recently in the treatment of human glioma (10–12). The most noteworthy effort to date uses polyl/(p-carboxyphenoxy)propane-sebacic acid (PCPP-SA) copolymer disks that release 1, 3-bis[2-chloroethyl]-1-nitro urea (BCNU). Following an extensive series of preclinical (13 for a review) and clinical studies (7–10), FDA approval was recently granted for the use of BCNU-loaded polymer disks 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. Using this approach, statistically significant patient benefit has been observed with median survival increased by 8 weeks (10).

Polymer devices such as disks can only be used in situations where the tumor is surgically accessible to create a cavity. They cannot be applied when tumors are located in surgically inaccessible portions of the brain, or are too numerous. Without the ability to surgically remove these tumors or deliver therapeuti c levels of chemotherapy to them, the tumor grows and the patient inevitably dies. If adequate concentrations of antineoplastic drugs could be delivered directly to these normally untreated tumors, greater patient benefit might be observed. One means of permitting delivery directly to the site of normally inoperable tumors is the use of polymeric microspheres. Microspheres can be formulated to provide excellent in vivo release kinetics, delivering high local concentrations of drugs for prede fined periods of time ranging from days to months. Microspheres have been proven to be efficient systems for delivery of a wide range of chemotherapeutic drugs (12–14) and can be easily injected as a suspension allowing drug delivery into virtually any site of the brain with minimal invasiveness (15,16).

Using an animal model of surgically resected glioma, we previously reported the first direct evidence that injections of sustained release microspheres into the tissue surrounding the tumor cavity provide superior survival effects over that obtained with injections into the cavity (17). Injections of sustained release microspheres into the tissue surrounding the resection cavity were intended to overcome the limited diffusion of drugs within brain tissue, allowing the tumor to be treated both locally and in regions of likely tumor infiltration. This approach might also represent a significant advance in the ability to treat inoperable tumors, since the microspheres could easily be injected into either the tumor itself and/or into the tissue surrounding...
the tumor. The following experiments are the first to evaluate the potential value of sustained release chemotherapy into the tissue surrounding a growing inoperable tumor mass versus into the tumor itself. Direct comparisons are made between sustained release formulations within the tumor and into the tumor perimeter, relative to equipotent bolus injections into both sites. The efficacy produced by two different chemotherapeutic drugs, carboplatin and BCNU, was tested in this model by injecting the microspheres either directly into the tumor or into the tissue along the perimeter of the tumor, and monitoring the animals for survival. The results extend prior observations following surgical resection of glioma (17), while providing the first evidence for the superiority of peritumoral injections over direct injections into a deep inoperable tumor as a means of interstitial chemotherapy. If the superiority of peritumoral over direct tumoral chemotherapy is confirmed in human glioma trials, the future use of polymeric delivery systems for treating brain tumors could be dramatically altered.

MATERIALS AND METHODS

Subjects

Male Fischer rats (N = 341; 200–220 g; Taconic Farms, Germantown, NY) were used in the following 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 (18). Rats were anesthetized using an intramuscular injection of a solution containing 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 with a 22 gauge needle, RG2 cells were injected unilaterally into the striatum (1 × 10³ cells/5 μl) at the following coordinates; A-P (+2.0 mm), L (+3.0 mm) and V (−6.5 mm) (19).

Fabrication of Carboplatin- and BCNU-Loaded Microspheres

PLG microspheres were fabricated for sustained release of carboplatin and BCNU as previously described (17). Carboplatin-loaded (Sigma Chemical) microspheres (PLG, Microsorb 50/50 DL, MW = 10 kD, Alkermes Inc., Wilmington, Ohio) were fabricated by a coacervation process with a carboplatin loading density of 10% (w/w). BCNU-loaded (Sigma Chemical) microspheres were fabricated by a solvent evaporation process with a loading density of 15% (w/w).

Survival Following Implantation of Carboplatin-Loaded Microspheres

An initial study characterized the survival benefit produced by sustained delivery of carboplatin in animals bearing small, 3 day old tumors. RG2 cells were implanted unilaterally into the striatum and 3 days later, the same animals received injections of microspheres containing carboplatin, or a bolus injection of carboplatin, directly into the center of the tumor at the same coordinates used to implant the RG2 cells. 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). Identical amounts of microspheres were injected in all cases by adding blank microspheres to the suspension. Microspheres (1 mg/10 ul) were stereotaxically injected at a rate of 2 ul/minute using a 10 ul Hamilton syringe with an attached 23 gauge needle. Animals were assigned to one of 5 treatment groups: (1) no treatment (n = 15), (2) a bolus injection of 100 μg of carboplatin, (n = 12), (3) 10 μg sustained release carboplatin (n = 10), (4) 50 μg sustained release carboplatin (n = 13), or (5) 100 μg sustained release carboplatin (n = 16).

A second series of experiments examined the effects of sustained release carboplatin on larger, 8 day old striatal tumors. Eight days following tumor implantation, rats were assigned to one of 4 treatment groups: (1) 100 μg carboplatin as a bolus (n = 10), (2) 10 μg sustained release carboplatin (n = 12), (3) 50 μg sustained release carboplatin (n = 13), or (4) 100 μg sustained release carboplatin (n = 10). All injections were made directly into the center of the tumor at the same coordinates used for implantation of the RG2 cells.

These experiments also directly compared the survival produced by sustained release following implantation of microspheres directly into the center of the tumor vs the tissue along the perimeter of the tumor. For implantation of the microspheres into the tissue along the perimeter of the tumor, animals received the same total amount of sustained release carboplatin that was delivered directly into the tumor, except that it was equally divided into 3 separate 3.3 ul aliquots. Eight days following tumor implantation animals were assigned to one of 4 treatment groups: (1) 100 μg carboplatin (33.3 μg/site) as a bolus (n = 19), (2) 10 μg (3.3 μg/site) sustained release carboplatin (n = 20), (3) 50 μg (16.7 μg/site) sustained release carboplatin (n = 22), or (4) 100 μg (33.3 μg/site) sustained release carboplatin (n = 22). Implants were made into 3 sites at the following coordinates along the perimeter of the tumor: A-P (+2.85 mm), L (+3.0 mm) and V (−6.5 mm); A-P (+1.15 mm), L (+2.0 mm) and V (−6.5 mm); A-P (+1.15 mm), L (+4.0 mm) and V (−6.5 mm). These coordinates were derived from prior studies in glioma and were calculated to place the microspheres approximately 0.5 mm outside of the tumor perimeter (20).

Survival Following Implantation of BCNU-Loaded Microspheres

A second series of experiments examined the effects of sustained release BCNU in animals bearing larger, 8 day old striatal tumors. Eight days following tumor implantation, rats were assigned to one of 5 treatment groups. For direct injections into the center of the tumor, animals received either (1) no treatment (n = 15), (2) a bolus injection of BCNU (n = 15), (3) 10 μg sustained release BCNU (n = 15), (4) 50 μg sustained release BCNU (n = 15), or (5) 100 μg sustained release BCNU (n = 15). Again, all injections were made directly into the center of the tumor at the same coordinates used for implantation of the RG2 cells.