LOCAL IMMUNOTHERAPY WITH INTERLEUKIN-2 DELIVERED FROM BIODEGRADABLE POLYMER MICROSPHERES COMBINED WITH INTERSTITIAL CHEMOTHERAPY: A NOVEL TREATMENT FOR EXPERIMENTAL MALIGNANT GLIOMA

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OBJECTIVE: Local delivery of carmustine (BCNU) from biodegradable polymers prolongs survival against experimental brain tumors. Moreover, paracrine administration of interleukin-2 (IL-2) has been shown to elicit a potent antitumor immune response and to improve survival in animal brain tumor models. We report the use of a novel polymeric microsphere delivery vehicle to release IL-2. We demonstrate both in vitro release of cytokine from the microspheres and histological evidence of the inflammatory response elicited by IL-2 released from the microspheres in the rat brain. These microspheres are used to deliver IL-2, and biodegradable polymer wafers are used to deliver BCNU, directly at the site of an intracranially implanted glioma in the rat. The two agents administered locally show a synergistic effect.

METHODS: Fischer 344 rats challenged intracranially with 9L gliosarcoma received an intracranial implant of either empty microspheres or microspheres containing IL-2 (IL-2 MS). Five days later, animals in each group were randomized to receive polymer implants loaded with 0, 3.8, or 10% BCNU at the tumor site.

RESULTS: Animals that received the combination of IL-2 MS and 3.8% BCNU polymer (median survival, 28.5 d) or IL-2 MS and 10% BCNU polymer (median survival, 45.5 d) showed significantly improved survival compared with animals that received monotherapy with IL-2 microspheres (median survival, 24 d), 3.8% BCNU polymer (median survival, 24 d), or 10% BCNU polymer (median survival, 32.5 d). Control animals had a median survival of 18 days. The combination of either 3.8 or 10% BCNU polymer with IL-2 MS resulted in 7 and 25% long-term survivors, respectively.

CONCLUSION: By showing synergy of IL-2 and BCNU in an animal glioma model and using a reproducible synthetic delivery system for each agent (i.e., one that did not rely on genetically engineered cells or viruses), we hope that the combination of local immunotherapy and chemotherapy can take an important step closer to clinical application in patients with malignant brain tumors.

KEY WORDS: Biodegradable polymer, Brain tumors, Cytokine, Interleukin-2, Interstitial chemotherapy, Microspheres

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The development of implantable polymers that release chemotherapeutic agents directly into the central nervous system (CNS) has had an impact on brain tumor therapy (4, 5, 35, 38, 39). This technology makes it possible to achieve high local concentrations of anticancer agents while minimizing systemic toxicity. In addition, it circumvents the need for the drug to cross the blood-brain barrier. Nitrosoureas, including carmustine (BCNU), have been used widely for the treatment of patients with malignant gliomas. Marginal efficacy combined with severe toxicity, however, has reduced the widespread use of systemic BCNU as an effective antiglioma agent (37). Local drug delivery by

chemotherapy-impregnated polymers implanted directly at the tumor site has provided a novel approach to malignant brain tumor therapy (3). With BCNU-loaded biodegradable polymers, this strategy has shown efficacy against experimental intracranial tumors (32) and has now demonstrated, in Phase III clinical trials, a statistically significant improvement in 6-month survival with human primary brain tumors, both at recurrence (5) and at initial presentation (35, 39).

The identification and cloning of genes encoding cytokines provided the impetus for another promising strategy in the treatment of brain tumors involving the stimulation of a local immune response directed against tumor cells (9, 11, 36). Cytokines such as interleukin-2 (IL-2) have no direct cytotoxic effect on neoplastic cells. They exert their immunomodulatory activity in a paracrine fashion whereby cytokines directly adjacent to potential tumor antigens can produce a strong local inflammatory response specific to the particular cytokine (23). Indeed, allogeneic cells genetically modified to secrete IL-2 have been shown to significantly prolong survival in nonimmunized mice when administered intratumorally (13, 19). IL-2 production by transduced cells is thought to bypass T-helper function in the generation of an antitumor response (8), thereby modulating the host's immunological response to the neoplasm. IL-2 is also necessary for cytotoxic T lymphocyte growth and enhances natural killer/leukocyte-activated killer cell activity (16, 23). We previously used genetically engineered autologous cells to release this cytokine in a paracrine fashion, and this resulted in potent antitumor responses in experimental brain tumor models (33). More recently, we showed that intracranial IL-2 paracrine therapy establishes immunological memory, protecting animals from tumor rechallenge in the brain and outside the CNS (7).

In an attempt to capitalize further on these two novel strategies, we hypothesized that the combination of paracrine immunotherapy and local delivery of chemotherapy by biodegradable polymers might act synergistically in a rat glioma model. We previously showed in a murine metastatic brain tumor model that combination treatment using nonreplicating genetically engineered tumor cells that produce IL-2, together with locally delivered chemotherapy, significantly improved survival compared with either therapy used alone (28). This synergy is likely due to both increased immunogenicity of tumor and abrogation of tumor-derived T-cell suppressor factors after exposure to cytotoxic drugs (22, 26, 34). In anticipation of potential clinical applications, we had two primary goals in performing this study: 1) to assess the efficacy of combined chemoimmunotherapy in a primary malignant glioma model and 2) to demonstrate that polymeric microspheres provide an effective, novel delivery vehicle for cytokines. The delivery of cytokines locally from microspheres has several advantages over cell-mediated delivery. Microsphere synthesis is less arduous and less labor-intensive than genetically modifying tumor cells. The production of microspheres and the pharmacokinetics of IL-2 release in vitro and in vivo are much more predictable; in addition, the likelihood of cell rejection by the host's native immune system is eliminated if an inert polymer is used for cytokine delivery. In this article, we report the initial animal study of the combined therapeutic efficacy of paracrine immunotherapy with IL-2 released from microsphere matrices and interstitial chemotherapy with BCNU released from polymer wafers to treat intracranial 9L gliosarcoma.

MATERIALS AND METHODS

Study Design

IL-2 was encapsulated into injectable microspheres composed of a biodegradable gelatin:chondroitin sulfate polymer (IL-2 MS) as previously described (14). The release of IL-2 from microspheres in vitro was determined. We then tested the effects and fate of IL-2 MS when stereotactically injected into the rat brain in comparison with microspheres loaded with control medium. After stereotactically implanting 9L glioma cells into the left parietal lobes of Fischer 344 rats, we tested the efficacy of local paracrine intracranial immunotherapy using IL-2 MS, with and without subsequent locally delivered BCNU.

Animals

One hundred eight 10-week-old female Fischer 344 rats weighing 180 to 220 g were used as indicated below. These animals were purchased from Charles River Laboratories (Wilmington, MA), maintained in standard animal facilities with three or four rats per cage, and given free access to rat chow and water. They were housed in accordance with the policies and principles of laboratory care of The Johns Hopkins University School of Medicine Animal Care and Use Committee.

Tumor Line

The 9L wild-type (9L wt) gliosarcoma cell lines (kindly provided by Dr. K. Plate, Department of Neuropathology, Freiburg University, Freiburg, Germany) were maintained in tissue culture in Dulbecco's minimum essential medium with 10% fetal bovine serum, streptomycin (80.5 pg/ml), penicillin (base, 80.5 U/ml), and 1% L-glutamine (all products from Gibco Laboratories, Grand Island, NY). Cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C. The cells were grown to confluence, detached with 0.25% trypsin in Dulbecco's phosphate-buffered saline, and resuspended in medium.

IL-2 Microspheres

IL-2 obtained from Chiron Corp. (Emeryville, CA) was encapsulated in polymeric matrices by the complex coacervation of gelatin (Atlantic Gelatin, Woburn, MA) and chondroitin 6-sulfate (Sigma Chemical Corp., St. Louis, MO) in the presence of IL-2 (14). Briefly, 3 ml of a 4% gelatin solution in distilled water at 37°C was mixed with 1 mg lyophilized IL-2 dissolved in 3 ml 0.2% chondroitin sulfate in phosphate-buffered saline at room temperature. Coacervation was achieved by the addition of the gelatin solution to a rapidly

mixed IL-2 and chondroitin sulfate solution. Microspheres were cross-linked with glutaraldehyde for 20 minutes and then poured into 10 ml of 0.1 mol/L aqueous glycine solution to stop the cross-linking reaction and quench the excess aldehyde groups. Cross-linked microspheres were collected by centrifugation and washed with phosphate-buffered saline. Placebo microspheres were prepared identically but without IL-2.

Cytokine Release Kinetics

IL-2 release from the gelatin-chondroitin sulfate microspheres (*Fig. 1*) was evaluated by performing an enzyme-linked immunoassay purchased from R&D Systems, Inc. (Minneapolis, MN). Briefly, IL-2 microspheres were equally distributed into eight vials containing a solution of 0.04 μmol/L collagenase in phosphate-buffered saline. The vials were capped to prevent evaporation and placed in an incubator at 37°C. For each time point (Days 1, 2 ,4,7, 19, 38, and 51), one of the vials was centrifuged, and the supernatant fraction was collected. The recovered solutions were assayed for the presence of IL-2.

Effects and Fate of Intracerebrally Injected Microspheres

To assess the fate of the microspheres after their intracerebral implantation, 24 rats were divided into 2 groups of 12 animals each and underwent left parietal stereotactically guided injection of 2 μl of a solution containing either empty or IL-2-loaded microspheres. Coordinates for the injection site were 3 mm lateral and 1 mm posterior to the bregma. Two animals from each group were killed on Day 1, 2, 4, 7, or 14. Two additional animals from each group were killed, one from each group on Days 21 and 28. Brains were harvested and fixed in 10% formalin. Tissue was embedded in paraffin, and 8-μm sections were stained with hematoxylin and eosin according to standard procedures.

BCNU Polymer Preparation

BCNU was obtained from Bristol-Myers-Squibb (Princeton, NJ), and polyanhydride poly[1,3-bis (carboxyphenoxy) propane-co-sebacic-acid] (CPP-SA) with a 20:80 molar ratio was supplied by Guilford Pharmaceuticals, Inc. (Baltimore, MD). CPP-SA polymers containing BCNU at 0, 3.8, and 10% loading by weight were prepared as described previously (30). Polymers for implantation were then pressed into disc shapes weighing 10 mg each and measuring 3 mm in diameter and 1 mm in height. We previously showed that these polymers release BCNU for a 2-week period (28, 30).

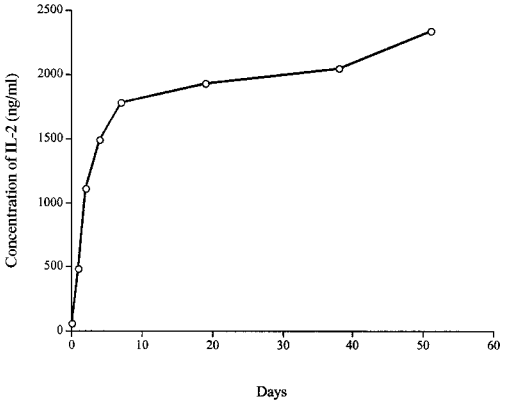

FIGURE 1. *Release kinetics of IL-2 from microspheres.*

Intracerebral Implantation of Microspheres and Tumor Cells

Rats were anesthetized with an intraperitoneal injection of a stock solution containing 25 mg/ml ketamine hydrochloride, 2.5 mg/ml xylazine, and 14.25% ethanol and diluted with 0.9% NaCl solution. The surgical site was shaved and prepared with a 70% ethanol- and iodine-containing solution. After a midline incision, a 3-mm burr hole was made 1 mm posterior to the coronal suture and 3 mm lateral to the sagittal suture. The animals were then placed into a stereotactic frame, and either microspheres alone (fate of microspheres study) or a mixture of the tumor cells and microspheres (efficacy study) were delivered for 15 minutes through a 26-gauge needle inserted to a depth of 3.0 mm at the center of the burr hole. The needle was then removed, the site was irrigated with 0.9% NaCl solution, and the wound was closed with surgical clips.

Polymer Implantation

The surgical incision used for inoculating the tumor was reopened 5 days later, and a single polymer was inserted into the cortex entirely below the level of the inner table of the parietal bone. After hemostasis was achieved, the placement site was irrigated and closed with surgical clips.

Efficacy Studies

Eighty-four animals divided into six groups initially underwent intracranial injection of 10^4 9L gliosarcoma cells. Groups 1, 3, and 5 received a co-injection of 9-μl placebo microspheres, followed by implantation on the fifth postoperative day of blank CPP-SA polymer (Group 1 [control animals], n = 15), 3.8% BCNU polymer (Group 3 [3.8% BCNU alone], n = 14), or 10% BCNU polymer (Group 5 [10% BCNU alone], n = 15). Groups 2, 4, and 6 received a co-injection of 9-μl IL-2 microspheres, followed by implantation on the fifth postoperative day of blank CPP-SA polymer (Group 2 [IL-2 microspheres alone], n = 14), 3.8% BCNU polymer (Group 4 [IL-2 MS/3.8% BCNU combination], n = 14), or 10% BCNU polymer (Group 6 [IL-2 MS/10% BCNU combination], n = 12).

Outcome and Statistical Analysis

The primary statistical outcome for all efficacy studies was time until death measured from the time of tumor implantation. All animals were monitored for any signs of neurotoxicity, and autopsies were performed, when possible, to confirm that death was a result of intracranial tumor. Survival distributions were estimated by using the product-limit method

(17). Differences between survival distributions were assessed in two stages. First, an overall test of heterogeneity among treatment groups was performed with the log-rank test (21). If this test rejected the groups (i.e., if treatment differences were present), pairwise comparisons between the treatment groups and the controls were performed with the log-rank statistic. All probability values reported are two-sided.

RESULTS

IL-2 Encapsulation and Release Kinetics from Polymer Microspheres

Polymer microspheres obtained by complex coacervation of positively charged gelatin with negatively charged chondroitin-sulfate in the presence of IL-2 were spherical and averaged 10 pm in diameter. Encapsulation efficiency was approximately 88.5%. Release kinetics of IL-2 from microspheres was characterized by a high rate of constant release during the first 7 days, followed by a slower, sustained release for the next 6 weeks (*Fig. 1*). The total dose of IL-2 from polymer microspheres was approximately 2.4 μg for 3 weeks, or 0.1 μg/d (14).

In Vivo Assessment of Effects and Fate of Microspheres Injected into Rat Brain

We found that after intracerebral injection, IL-2 MS elicited a strong, long-lasting, inflammatory infiltrate in the region surrounding the implantation site. This infiltrate was characterized by a marked predominance of polymorphonuclear leukocytes on Day 1 (*Fig. 2A*) and gradually evolved to a predominantly mononuclear infiltrate by Day 2 (*Fig. 2, B* and *C*). On Day 21, a few IL-2 MS were still visible within an area of mononuclear infiltrate corresponding to the injection site (*Fig. 2D*). These findings are similar to those seen when genetically engineered cells producing IL-2 were injected intracranially (28). In contrast, the injection of blank microspheres failed to elicit any kind of inflammatory infiltrate (*Fig. 3, A–C*). On Day 21, a few blank microspheres without surrounding inflammation were still visible (*Fig. 3C*).

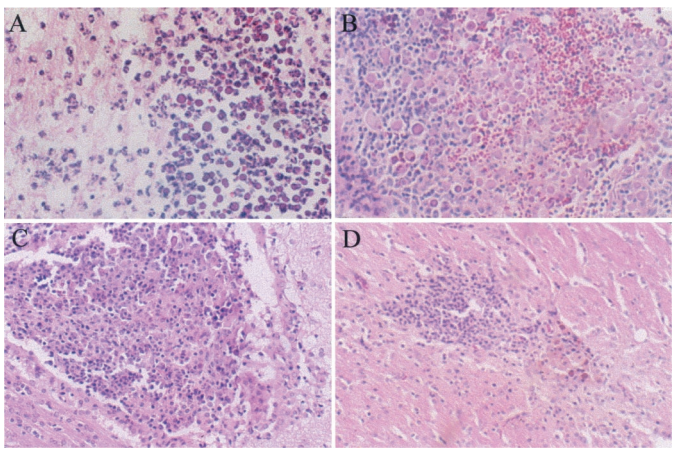

FIGURE 2. *Photomicrographs of stereotactically injected IL-2 microspheres within the rat brain.* A, *24 hours after injection there is a prominent polymorphonuclear leukocyte infiltrate.* B, *by 48 hours, this infiltrate has become primarily mononuclear.* C, *by Day 14, there is a heavy mononuclear leukocyte infiltrate.* D, *infiltrate persists at Day 21. Note the remaining microspheres.*

Efficacy Studies

All animals injected with 9L wt cells and blank microspheres followed by implantation of blank CPP-SA polymer (Group 1 [control group], n = 15) died, with a median survival time of 18 days (range, 15–24 d). All rats injected with a mixture of 9L wt cells and IL-2 MS followed by the implantation of blank CPP-SA polymer (Group 2 [IL-2 MS/blank polymer], n = 14) died, with a statistically significant prolonged survival compared with the control group (median, 24 d; range, 17–32 d; log-rank, $P < 0.0007$). All rats injected with 9L wt cells and blank microspheres followed by the implantation of 3.8% BCNU CPP-SA polymer (Group 3 [blank MS/3.8% BCNU], n = 14) died, with a statistically significant prolongation of survival compared with the control group (median, 24 d; range, 18–28 d; log-rank, $P < 0.0002$). All rats injected with 9L wt and blank microspheres followed by the implantation of 10% BCNU CPP-SA polymer (Group 5 [blank MS/10% BCNU], n = 15) died, with a statistically significant prolongation of survival compared with the control group (median, 32.5 d; range, 21–67 d; log-rank, $P < 0.0001$). Animals injected with 9L wt cells and IL-2 microspheres followed by the implantation of 3.8% BCNU CPP-SA polymer (Group 4 [IL-2 MS/3.8% BCNU combination], n = 14) survived significantly longer than the control group animals and also had a statistically significant prolongation of survival compared with groups that received either treatment alone (median, 28.5 d; range, 24–180 d; log-rank, $P < 0.0089$ versus IL-2 alone; log-rank, $P < 0.0001$ versus 3.8% BCNU alone). One animal from this group survived for more than 180 days (*long-term survivor;* i.e., survival for longer than 180 d). Animals that received intracranial 9L wt cells and were treated with the combination of IL-2 MS and 10% BCNU CPP-SA polymer (Group 6 [IL-2 MS/10% BCNU combination], n = 12) also had a statistically significant prolongation of survival compared with the two groups that received either treatment alone (median, 45.5 d; range, 18–180 d; log-rank, $P < 0.0001$ versus IL-2 MS alone; log-rank, $P < 0.02$ versus 10% BCNU alone), with 25% long-term survivors (*Fig. 4*, *Table 1*).

Neurotoxicity and Autopsy Findings

Animals that received the 9L tumor without any treatment had large, solid tumor masses at the injection site. Autopsies performed in treatment animals showed widespread intracranial tumor, confirming death occurred as a result of the tumor. There was no associated toxicity seen with the microsphere or the polymer implants. Long-term survivors were killed after Day 180 and were examined histologically. No signs of tumor growth were present in these animals.

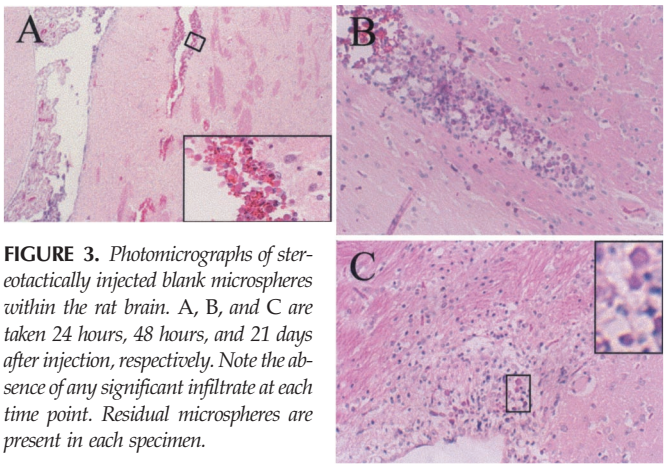

FIGURE 3. *Photomicrographs of stereotactically injected blank microspheres within the rat brain.* A, B, *and* C *are taken 24 hours, 48 hours, and 21 days after injection, respectively. Note the absence of any significant infiltrate at each time point. Residual microspheres are present in each specimen.*

DISCUSSION

In this study, we have demonstrated that the combination of intracranial paracrine immunotherapy with IL-2 delivered from synthetic polymer microspheres and local delivery of chemotherapy from biodegradable polymer wafers significantly improves survival in an experimental rat glioma model as compared with either antitumor therapy alone. Importantly, this synergistic effect yielded a high percentage of long-term survivors (25% in the IL-2 MS/10% BCNU combination group) against an otherwise lethal dose of intracranial tumor. Histological examination showed the microsphere matrices to be inert, with no overt CNS toxicity.

Given the poor prognosis of patients with malignant brain tumors and the high incidence of local recurrence after treatment, efforts to improve survival have focused on controlling local disease. One significant hindrance in this effort has been the unique environment of the CNS. Chemotherapeutic agents, immune response modifiers (e.g., cytokines), and other anticancer therapies are often unable to cross the blood-brain barrier. Furthermore, significant systemic toxicity can result from the administration of these agents in doses large enough to achieve efficacious concentrations in the brain. To overcome these limitations, strategies for the local delivery of therapeutic agents have gained increasing attention.

The introduction of implantable polymers that release chemotherapeutic agents directly into the CNS is an example of a strategy that has had an impact on glioma therapy (3, 38). This technology circumvents the need for a drug to cross the blood-brain barrier, making it possible to achieve high local concentrations of anticancer agents while minimizing systemic toxicity. Nitrosoureas, including BCNU, are a class of chemotherapeutic agents that have been used widely for the treatment of patients with malignant gliomas (2, 18, 37). Because of their relative lipid solubility and low molecular weight, these agents can penetrate the blood-brain barrier when administered intravenously and reach tumoricidal concentrations in the brain. However, the systemic side effects, which include myelosuppression and pulmonary fibrosis, can be severe and have limited the use of systemic nitrosoureas for the treatment of malignant brain tumors. To combat these systemic effects, BCNU has been incorporated successfully into biodegradable polymers and has shown some short-term efficacy in clinical trials in patients with both recurrent (4, 5) and newly diagnosed (35) malignant brain tumors. Moreover, this treatment has not been associated with any systemic side effects.

More recently, immunotherapeutic techniques have been developed as an antineoplastic strategy against brain tumors (12, 13, 19, 20, 24, 25, 31, 33). Initial efforts focused on isolating elements of the host immune system, exposing them to antigen and cytokine, and then readministering these immune effectors in a systemic fashion in the hope of generating specific antitumor responses. Unfortunately, these systemic approaches were hampered by several factors, including the blood-brain barrier, which tightly restricts the passage of immune effector cells across brain epithelium (15), poor target recognition within the central nervous system, immune resistance and other protective mechanisms intrinsic to brain tumors (10, 29), and limited expression of major histocompatibility antigens by tumor cells in the brain (1).

Although we report that no evidence of edema was found in the experimental animals, we do note that humans and rodents have significantly different tolerances to cytokines. Slow release of IL-2 through a controlled-release polymer system in humans is likely to reduce toxicity and edema. We previously showed this effect in comparing the delivery of chemotherapy by intracranial polymer implants with systemic delivery (5, 32).

The identification and cloning of genes that encode specific cytokines has provided an important advance in the ability to activate immunological effector responses in the brain. Because cytokines exert their immunomodulatory effect in a paracrine fashion local to the site of antigen, cells that are genetically engineered to secrete a specific cytokine can be placed directly into the CNS, thus bypassing limitations posed by the systemic administration of cytokines (23). Furthermore, this paracrine biology more closely resembles the natural biology of cytokines and allows for high concentrations directly at the site of tumor antigens. By using the strategy of ex vivo gene transfer, we previously showed that paracrine intracranial delivery of cytokines, such as IL-2 and IL-12, with transduced autologous tumor cells is highly effective in treating experimental brain tumors (6, 33). In addition, we showed that combination treatment with nonreplicating, genetically engineered tumor cells that produce IL-2, together with locally delivered chemotherapy, significantly improved survival over either therapy used alone in a murine metastatic brain tumor model (28).

Nevertheless, several practical considerations may limit the use of cells genetically engineered to secrete cytokines for the treatment of patients with brain tumors. The gene transduction process is not uniformly successful and can require up to several months to be completed. Transduced cells must then be selected and reimplanted, necessitating a second operation. In addition, in vivo gene therapy models that use viral vectors

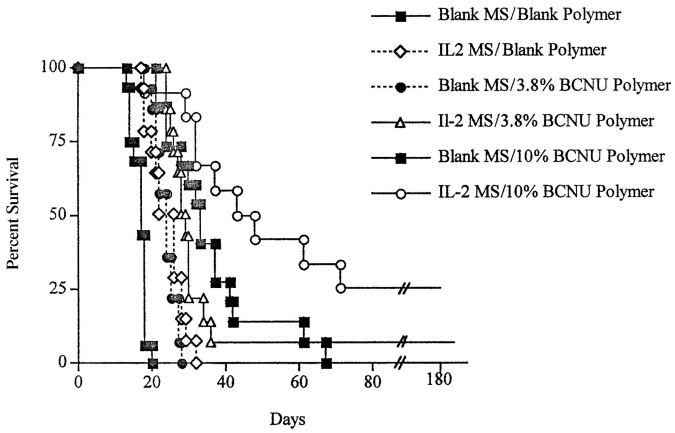

FIGURE 4. *Kaplan-Meier survival curves for rats challenged with a lethal intracranial dose of 9L gliosarcoma cells and treated with either blank microspheres and blank polymer, IL-2 MS and blank polymer, blank microspheres and 3.8% BCNU polymer, IL-2 MS and 3.8% BCNU polymer, blank microspheres and 10% BCNU polymer, or IL-2 MS and 10% BCNU polymer.*

encoding cytokine genes may be limited by the low efficiency of in vivo gene transfer (27). Thus, in the present study, we have used a biodegradable polymer microsphere system for the local delivery of cytokines that avoids these difficulties. As a result, we have broadened this novel treatment strategy of combining chemo- and immunotherapeutic agents by making two important preclinical advances. First, we have demonstrated the synergy of local IL-2 and BCNU in a primary malignant brain tumor model. When used in combination against the 9L gliosarcoma, local IL-2 immunotherapy and interstitial BCNU chemotherapy significantly improved survival over either antitumor agent alone and also yielded a significant number of long-term survivors. Second, we have shown the efficacy of a novel synthetic microsphere system that is based on the use of polymers that are capable of long-term IL-2 delivery after a single administration. The use of IL-2 microspheres enables the delivery of high concentrations of cytokine in a controlled fashion at the tumor site while avoiding the restriction imposed by the blood-brain barrier and minimizing systemic toxicity.

CONCLUSIONS

This study demonstrates a novel approach to intracranial paracrine immunotherapy combined with interstitial chemotherapy in the treatment of brain cancer. The potential of enhancing the intrinsic immunogenicity of malignant brain tumors with cytotoxic agents may provide an avenue for the effective use of immunotherapy against CNS neoplasms and may prove to be a powerful means of eradicating residual disease after surgical resection. The use of implantable, synthetic, biodegradable vehicles for the delivery of therapeutic agents not only will circumvent the problems associated with biological systems (i.e. transduced cells or viral vectors) and the limitations imposed by the blood-brain barrier but also will enable the delivery of immunotherapy and chemotherapy in high concentrations at the site of tumor in a predictable, reproducible manner, producing synergistic benefit in treating malignant brain tumors.

TABLE 1. Efficacy of local interleukin-2 immunotherapy with or without interstitial carmustine polymer chemotherapy[a]

Group	No. of animals	Median survival (d)	Survival range (d)	LTS[b] (%)	*P* value[c]
1 (blank MS/blank polymer [control])	15	18	15–24	0	—
2 (IL-2 MS/blank polymer)	14	24	17–32	0	<0.0007 versus control
3 (blank MS/3.8% BCNU)	14	24	18–28	0	<0.0002 versus control
4 (IL-2 MS/3.8% BCNU)	14	28.5	24–180	7%	<0.0001 versus control <0.0089 versus IL-2 MS alone <0.0001 versus 3.8% BCNU alone
5 (blank MS/10% BCNU)	15	32.5	21–67	0	<0.0001 versus control
6 (IL-2 MS/10% BCNU)	12	45.5	18–180	25%	<0.0001 versus control <0.0001 versus IL-2 MS alone <0.02 versus 10% BCNU alone

[a] LTS, long-term survivor; MS, microsphere; IL-2, interleukin-2; BCNU, carmustine.
[b] Long-term survivor defined as living more than 180 days.
[c] Significance values were calculated by using the log-rank (Mantel-Cox) test.

DISCLOSURE

Under a licensing agreement between Guilford Pharmaceuticals and the Johns Hopkins University, Dr. Brem is entitled to a share of royalty received by the University on sales of products described in this work. Dr. Brem and the University own Guilford Pharmaceuticals stock, which is subject to certain restrictions under University policy. Dr. Brem also is a paid consultant to Guilford Pharmaceuticals. 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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COMMENTS

On this article, the authors used the 9L gliosarcoma model in Fischer rats as a way of testing local immunotherapy with interleukin-2 (IL-2) combined with interstitial chemotherapy with carmustine (BCNU). The authors demonstrate that treatment with IL-2-containing microspheres in combination with polymers containing 3.8% or 10% BCNU significantly increased survival; indeed, there was 25% long-term survival among animals treated with high-dose BCNU in combination with the IL-2 microspheres. This study suggests that the use of immunotherapy with IL-2 and locally delivered BCNU may be efficacious in human tumors. Clearly, this study is inadequate for leading to human studies at this time. The study entails the usual problems encountered with using rodent models. The tumors are quite small, and thus, it is easy to get a diffusion effect because of the distance of the tumor. A human tumor is larger than the entire rat's brain; thus, it is difficult to determine whether this treatment will be effective in a large-volume tumor model. Hopefully, the authors will pursue it, and it will lead to efficacious therapy in the human setting.

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With their latest work, the authors expand their previous research on the optimization of local chemotherapy to gliomas. There is now evidence that more radical surgery for malignant glioma will translate into extended survival (2); therefore, eradication of tumor cells that remain after resection is a major, valid goal and becomes part of the intraoperative neurosurgical strategy. Since the local administration of BCNU in slow-release polymers has become a widely used adjunct to microsurgical resection, additional modalities need to be explored, because the effect of the local BCNU treatment is of 2 months' duration on average. It is a logical next step to embark on an immunological strategy, because it has been observed that wafer implants in humans are surrounded by some inflammatory reaction, mainly of a macrophage nature, while being degraded. We also know that the immediate milieu of high-grade gliomas is immunosuppressed; thus, enhancement of a possible local immune response seems promising and needs to be explored. By showing the feasibility and efficacy in an in vivo model, the authors have laid the groundwork for possible human trials.

It should be mentioned that this kind of intracavitary therapy, in which wafers are placed into the infiltrating zone, will have a chance to work only after the tumor is well resected, because the effect against solid tumor will be minimal. It should also be borne in mind that no intracranial model in rodents has yet been able to predict the behavior of agents in the human situation. We have experienced dramatic long-term toxicity from intracavitary administration of IL-2 in humans, although it was given in higher doses that resulted in the termination of the studies (1). For prolonged slow release of low doses, the toxicity can be expected to be different than for direct injection. Once such dose finding is accomplished, the combination therapy may extend the effect of presently available local intracavitary chemotherapy.

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The therapeutic potential of local delivery via biodegradable polymers is certainly worth exploring for the management of brain tumors. In a series of well-designed experiments, Rhines et al. show a synergistic effect of IL-2 and BCNU in the 9L gliosarcoma model in Fischer rats. Although interesting, this model has several limitations. First, it is unfortunately obvious that such models do not reflect the complexity of a human tumor with invasive properties. Second, as underlined by the authors, the toxicity of cytokines in the central nervous system is different in humans and rodents, leading to the sad conclusion that rodents are more convenient to treat. Finally, despite impressive statistical results, the survival difference between animals treated with BCNU alone and those treated with a combination of BCNU and IL-2 is only a few days, in a model designed to observe an effect. Thus, the direct extrapolation of these results to the human setting is unlikely. However, the data presented here should encourage the investigation of biodegradable polymers with other bioactive molecules.

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The authors describe the use of combining local immunotherapy with interstitial chemotherapy. The combination of intracranial paracrine immunotherapy with IL-2 delivered from synthetic polymer microspheres and local delivery of chemotherapy via a biode-

gradable polymer wafer is an interesting concept, and in this model, it did significantly improve survival over either antitumor therapy alone. However, the efficacy of this synergistic response cannot be easily translated to humans, particularly because the 9L model is essentially a gliosarcoma and is not truly representative of the human cerebral glioma.

Nevertheless, the concept is interesting. In particular, the use of a biodegradable polymer microsphere for the delivery of cytokines avoids many of the difficulties that limit the use of cells genetically engineered to secrete cytokines. In addition, the use of IL-2 microspheres overcomes some of the biological problems associated with IL-2 delivery, because the microspheres enable the delivery of high concentrations of cytokine to the tumor site, thus bypassing the local blood-brain barrier and minimizing systemic toxicity.

Although it is conceivable that this type of combined local therapy would improve local control of glioma and reduce local recurrence of the tumors, its effectiveness is likely to be limited owing to the inherent infiltrative nature of these tumors and the inability of a local therapy to influence this key biological factor.

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In this experimental study, the authors evaluated the potential synergistic effect of using a cytokine along with an alkylating agent to enhance cytotoxicity linked to increasing the immunogenicity of the tumor. This provides a novel strategy for so-called chemoimmunotherapy. The cytokine that was evaluated was IL-2, which produced an inflammatory response by enhancing cytotoxic T cell function, as well as stimulating natural killer/leukocyte-activated killer cell activity. This resulted in a brisk inflammatory response within the tumor, secondary to the slow release of IL-2 over a 6-week period. This was accomplished using a novel delivery strategy, i.e., polymer microspheres, which resulted in very acceptable release kinetics of the cytokine. The use of BCNU delivered through polymers in combination with the IL-2 slow-release microspheres resulted in improved survival in this animal system.

This is a very straightforward study that has important implications for the treatment of patients with brain tumors. For example, we know that alkylating agents alone, no matter how they are administered, will not result in long-term cures in the vast majority of patients with high-grade gliomas. Thus, the principle of synergism, using an immune-modulatory cytokine, is very attractive, especially if it can be delivered locally without being hampered in its effects by an intact blood-brain barrier in the brain adjacent to tumor. Thus, the authors demonstrate the synergistic effect of this chemoimmunotherapy, and they should carry this result forward to a Phase I clinical trial.

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