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Local Delivery of Chemotherapy and Concurrent External Beam Radiotherapy Prolongs Survival in Metastatic Brain Tumor Models[1]

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ABSTRACT

Local chemotherapy with biodegradable polymers prolongs survival with minimal morbidity in patients with intracranial high-grade gliomas. However, use of local chemotherapy for metastatic brain tumors has not been defined. We studied the safety and the efficacy of locally delivered chemotherapy with and without concurrent radiation therapy in treating tumors that frequently metastasize to the brain.

The chemotherapeutic agents 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), carboplatin, and camptothecin were incorporated into controlled-release polymers and tested individually against intracranial challenges with one of four tumors (lung carcinoma, renal cell carcinoma, colon carcinoma, and melanoma). For each combination of drug and tumor type, four groups were tested: (*a*) empty polymer (no drug); (*b*) external beam radiotherapy (XRT) alone; (*c*) local chemotherapy from biodegradable polymer alone; and (*d*) local chemotherapy and XRT together. Polymers were implanted 5 days after tumor inoculation; XRT was given on days 7–9 (300 cGy/day).

BCNU and XRT together were effective against all four tumors. BCNU polymer alone significantly prolonged survival in mice with intracranial melanoma or renal cell carcinoma. Carboplatin alone was effective against both melanoma and colon carcinoma and in combination with XRT against colon and renal cell carcinomas. Camptothecin was effective only with XRT against melanoma.

These studies demonstrate that local delivery of chemotherapy with concurrent radiation therapy is safe and can significantly prolong survival in models of common intracranial metastatic tumors. Concurrent use of local chemotherapy with standard XRT appears to be more effective than either treatment alone. Local chemotherapy may also be of benefit to patients who have previously received maximal cranial irradiation but suffer an intracranial recurrence.

INTRODUCTION

Metastatic lesions account for a majority of newly diagnosed intracranial tumors, are increasing in incidence, and are uniformly fatal if untreated (1). The current therapies available are only moderately successful in providing local control of intracranial tumor metastases. Improved local control of brain metastases is important for several reasons. First, many patients die of their intracranial disease despite aggressive therapy. Patients who present with metastases to the brain only are more likely to die of neurological complications than of systemic progression of their disease, despite aggressive therapy (2, 3). This is most dramatic for melanoma, in which up to 91% of patients who develop intracranial metastases die from CNS[4] rather than systemic disease (4–8). Second, as therapies aimed at treating systemic metastases improve, intracranial relapse may become more common, especially if these new therapies have poor CNS penetration. For example, in trials of both immunotherapy (9) and new chemotherapeutic regimens (10–12), patients have succumbed to CNS disease while they remain disease-free systemically. For these patients, control of disease in the brain may be the only obstacle to long-term survival. Third, improved local control in the CNS may enhance the quality of life even if overall survival is not prolonged (3, 13). For all these reasons, efforts to improve local control of brain metastases continue.

Currently, therapies used to control local growth of brain metastases include two local treatments, surgery and stereotactic radiosurgery, and two nonfocal treatments, external beam whole-brain radiotherapy and chemotherapy. Surgery has traditionally been considered the optimal therapy for patients with a single lesion (5, 14, 15) and perhaps for patients with two or three accessible lesions (16). The rationale for surgical therapy in the treatment of patients with brain metastases is that surgery aids in the control of local recurrence, prolongs survival, removes mass effect, and, when the diagnosis is unknown, provides tissue for pathological examination. In a series of patients with non-small cell lung cancer (the most common type of brain metastasis), complete surgical resection was the best predictor of survival (17). Despite surgical resection and XRT, however, 31–48% of patients with intracranial metastases suffer tumor recurrence in the CNS. Two-thirds of these recurrences are at the site of resection (3). Clearly, although surgical resection is beneficial to patients, additional strategies are needed to achieve local control of brain metastases.

Recently, efforts to achieve local control of brain metastases have been expanded to include stereotactic radiosurgery, delivery of a single dose of focused radiation to a well defined lesion. Although initial results are encouraging, local failure rates of 15–75% have been reported (18, 19), and the procedure is best for treating small lesions (<3 cm; Refs. 19–21). Finally, conventional external beam radiation therapy and chemotherapy have been used either alone or in combination with local therapies in treating brain metastases. At most centers, radiation therapy is routinely given in conjunction with surgery or stereotactic radiation (20, 22, 23), whereas the role of systemic chemotherapy for most metastatic intracranial tumors is unclear (22). Thus, current therapies for CNS metastases are not sufficient to prevent local recurrence at the resection site in many patients. For this reason, we explored methods of improving the local control of brain metastases without increasing the potential morbidity to the patient.

Several studies have established that intracranial chemotherapy with BCNU delivered by biodegradable polymer wafers is a safe and effective therapy for patients with malignant gliomas (24–26). Morbidity associated with the use of high-dose local chemotherapy is minimal (27). The role of this new treatment for metastatic brain tumors is unknown. We hypothesized that local chemotherapy, in conjunction with XRT, could be used to improve survival in experimental models of brain metastases. We then developed models of common histopathological types of brain metastases and systematically tested this hypothesis.

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[4] The abbreviations used are: CNS, central nervous system; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine); XRT, external beam radiotherapy; CPP, 1,3-bis-(*p*)-carboxyphenoxy propane; SA, sebacic acid; IQR, interquartile range.

MATERIALS AND METHODS

Overview. First, we developed murine models for brain tumor metastasis with four common tumor types (melanoma and lung, colon, and renal cell carcinomas). Second, we determined the maximum tolerated doses of locally delivered chemotherapy in the mouse brain with and without subsequent radiation therapy. Third, with the toxicity of the agents established for our mouse models, we then tested the efficacy of each agent against each tumor line with and without radiation therapy.

Tumor Lines. B16-F10 melanoma cells, Lewis lung carcinoma cells, and CT26 were obtained from the Division of Cancer Treatment Tumor Repository (National Cancer Institute, Frederick, MD) and are standard models of melanoma, lung carcinoma, and colon carcinoma, respectively. The RENCA cell line, a standard model for renal cell carcinoma, was graciously provided by Dr. Drew Pardoll (Johns Hopkins University School of Medicine, Baltimore, MD). The cells were maintained in DMEM containing 10% FCS and penicillin/streptomycin in humidified incubators gassed with 5% carbon dioxide. Cultured tumor monolayers were harvested with trypsin, counted, and resuspended in the medium prior to intracranial injection.

Animals. C57/Bl6 female mice used for B16 and Lewis lung carcinoma experiments and BALB-C female mice used for CT26 and RENCA experiments were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and were 6–12 weeks old. Each tumor line was placed in the mouse strain with which it is immunologically syngeneic. These animals were allowed free access to Baltimore city water and rodent chow; they were kept in accordance with the policies and principles of laboratory animal care of the Johns Hopkins University School of Medicine Animal Care and Use Committee.

Chemotherapeutic Agents. BCNU and *cis*-diammine-1,1-cyclobutane-dicarboxylate platinum(II) (carboplatin) were obtained from Bristol-Myers Squibb (Princeton, NJ). Sodium camptothecin was obtained from the National Cancer Institute (Bethesda, MD).

Polymers. The CPP:SA copolymer was synthesized from CPP and SA at a 20:80 molar ratio. Preparation of CPP:SA polymers containing either BCNU (28) or carboplatin (29) is described in detail elsewhere. Briefly, for BCNU and camptothecin, disc-shaped polymer implants (each 1.5 mm in diameter, 0.5 mm in height, and 5 mg in weight) were prepared by dissolving CPP:SA and the drug in methylene chloride. Because sodium camptothecin is poorly soluble in methylene chloride, single drops of methanol were added to the mixture until the sodium camptothecin was dissolved. The mixture was allowed to evaporate for at least 3 days in a desiccator. The polymers were then compression molded into their final shape. Polymers containing carboplatin were formed by mix melting CPP:SA and carboplatin. The mixture was allowed to cool, and a punch mold was used to create 5-mg disc-shaped polymers 1.5 mm in diameter and 0.5 mm in height.

Tumor Inoculation. For stereotactic intracranial injections of tumor cells, the surgical site was shaved and prepared with 70% ethyl alcohol and iodine-containing solution. After a midline incision, a 2-mm burr hole centered 2 mm posterior to the coronal suture and 2 mm lateral to the sagittal suture was made. Animals were then placed in a stereotactic frame and the tumor cells (total volume, 5 μl) were delivered by a 26 gauge needle inserted to a depth of 3 mm over a period of 3 min. The needle was removed, the site was irrigated with sterile 0.9% NaCl solution, and the skin was closed.

Polymer Placement. For assessing toxicity of drug-impregnated polymers in the absence of tumor, polymers were placed in the cortex entirely below the level of the inner table of the parietal bone through a newly drilled parietal burr hole. For assessing the efficacy, the surgical incision used for inoculating the tumor was reopened 5 days later, and the polymer was inserted as described above. Because no gross tumor was evident, tumor resection was not attempted. After hemostasis was confirmed, the site was irrigated with sterile 0.9% NaCl solution, and the skin was sutured.

Radiation Therapy. Prior to irradiation, the dose (cGy) to the intracranial tumor implants was calibrated using radiochromic dye medium (Gafchromic; Nuclear Associates, Carle Place, New York) mounted in polystyrene mouse phantoms. The change in absorbance *versus* dose was measured as described (30). For radiation treatments, mice were restrained in ventilated plastic centrifuge tubes with their heads positioned a fixed distance from the collimated 137-Cs source of a laboratory irradiator (Mark I irradiator, Model 68; J. L. Shepard and Associates, San Fernando, CA). Radiation was delivered at a dosage of 300 cGy/day (single fraction) for 3 consecutive days (total dose, 900 cGy).

Development of Tumor Models. For each tumor line, mice were inoculated with escalating doses of tumor cells in the left parietal lobe as described above under "Tumor Inoculation." Groups of five mice were given injections of either 10^2, 10^3, 10^4, 10^5, or 10^6 tumor cells. Survival was recorded, and autopsies were performed whenever possible to determine cause of death.

Toxicity Studies without Radiation. Prior to efficacy studies, the intracranial toxicity of BCNU, camptothecin, and carboplatin loaded in CPP:SA polymer in mice was determined without tumor. Animals underwent a single operation during which a left parietal burr hole was made as described above. For BCNU, groups of five mice underwent placement of BCNU-loaded polymer at either 0 (empty polymer), 5, 10, 15, or 20% loading by weight of BCNU. For carboplatin, groups of five mice underwent placement of carboplatin-loaded polymer at either 0.5, 1, or 3% loading by weight. For camptothecin, groups of six animals underwent placement of camptothecin polymer at either 2, 5, or 10% loading by weight. Animals were monitored for evidence of neurological complications (*e.g.*, lethargy and hemiparesis), and survival was assessed. The maximum nontoxic dose was defined as the dose that allowed at least 80% of the animals to survive for 100 days. After 100 days, mice in the maximum tolerated dose group were sacrificed, and their brains were histologically analyzed with standard H&E staining.

Toxicity Studies with Radiation. Once the maximum tolerated loading dose for each drug was determined, a second trial was performed for each drug during which concurrent XRT was given. Two days after polymer placement, whole-brain XRT (300 cGy per day for 3 successive days) was given as described under "Radiation Therapy." Drug loadings up to the maximum dose identified in the toxicity studies without XRT were tested. The following doses were tested in groups of five animals: BCNU, 0, 1, 5, 10, and 20%; carboplatin, 0.5, 1, and 3%; and camptothecin, 1, 3, 6, and 10%. Animals were monitored for evidence of neurological complications, and survival was recorded. The highest nontoxic dose was again defined as the maximal dose at which at least 80% of the animals survived 100 days. After 100 days, surviving mice in the group receiving the maximum tolerated dose were sacrificed, and their brains were analyzed histologically with H&E staining.

Histology. Two H&E sections per animals were analyzed in a blinded fashion by a pathologist (D. J. B.). Toxicity was systematically assessed in terms of neuronal injury, white matter damage, gliosis, inflammation, and necrosis. Neuronal injury, cytoplasmic eosinophilia, nuclear pyknosis, and neuronal dropout was graded in the CA1, CA2, and CA3 regions of the hippocampus and in the Purkinje cell layer of the cerebellum. White matter damage was assessed in the corpus callosum by grading edema, myelin vacuolization, and gliosis. Cerebral cortical gliosis was graded in the parietal cortex ipsilateral to the more affected hippocampus. Inflammation, necrosis, and hemorrhage were described and graded when present at any location within the histological section.

Efficacy Study. Twelve separate efficacy trials were performed, one for each combination of tumor line (melanoma, colon carcinoma, renal cell carcinoma, and lung carcinoma) and drug (BCNU, carboplatin, and camptothecin). The protocol and rationale for the four groups in each efficacy trial is described in Table 1. Briefly, mice were randomized into groups of 8–15 mice and underwent tumor cell inoculation. Five days later, polymers were placed. Radiation was given on days 7–9 following tumor inoculation. The four groups received the following treatments: (*a*) blank CPP:SA polymers (no drug) 5 days after tumor inoculation, no radiation (control group); (*b*) no polymer placement on day 5, radiation on days 7–9; (*c*) highest tolerated dose of polymer delivered chemotherapy on day 5 (dose determined in first toxicity trial without XRT), no radiation; and (*d*) highest tolerated dose of polymer delivered chemotherapy on day 5 (dose determined in second toxicity trial with XRT), radiation therapy on days 7–9.

Outcome Analysis. For all efficacy studies, survival was the primary end point. Animals were monitored for signs of clinical neurotoxicity such as hemiparesis or lethargy. In previous experiments in our laboratory, moribund or paralyzed mice were seen to die in 12–24 h; therefore, in these experiments, they were sacrificed. Whenever possible, animals were autopsied to determine the cause of death. The distribution of the intervals until death were estimated using the method of Kaplan and Meier (31). Comparison of survival between groups was made using two nonparametric statistical analyses, the Mann-Whitney *U* test and the Kruskal-Wallis test.

Table 1 *Treatment paradigm and rationale*

	Group 1, control	Group 2, radiation therapy	Group 3, maximal local chemotherapy	Group 4, local chemotherapy and radiation therapy
Day 0	Tumor placement	Tumor placement	Tumor placement	Tumor placement
Day 5	Blank polymer placed	No polymer placed	Maximum tolerated chemotherapy polymer placed[a]	Maximum tolerated chemotherapy polymer placed[b]
Day 7–9	No XRT	300 cGy/day for 3 days	No XRT	300 cGy/day for 3 days
Purpose of group	Confirm lethality of tumor	Investigate efficacy of XRT (current standard clinical treatment)	Investigate efficacy of local chemotherapy alone	Investigate likely clinical scenario, surgery with polymer placement followed by XRT

[a] A toxicity trial determined the maximum tolerated dose of chemotherapy without subsequent radiation therapy (BCNU, 20% loading by weight; carboplatin, 1%; and camptothecin, 10%).

[b] A separate toxicity trial determined the maximum tolerated dose of chemotherapy with subsequent radiation therapy (BCNU, 10% loading by weight; carboplatin, 0.5%; and camptothecin, 10%).

Table 2 *Development of intracranial tumor models for four tumor lines that frequently metastasize to the brain*

Tumor histology	No. of cells injected	Median survival (days)	Range of survival (days)
Lewis lung carcinoma	10,000	23	22–27
RENCA	10,000	23	17–24
CT26 (colon adenocarcinoma)	10,000	21	20–24
B16F10 (melanoma)[a]	100	18	16–20

[a] R. C. Thompson *et al.* (32).

RESULTS

Development of Tumor Models. Escalating doses of tumor cells from each line were stereotactically injected into the brains of syngeneic mice to define the intracranial tumor models. Table 2 shows the dose of colon, lung, and renal cell carcinoma cell lines that produced median survivals of 20–25 days following intracranial injection. The optimal dose of tumor cells in the B16 melanoma intracranial tumor model had previously been determined in our laboratory (32).

Toxicity of Radiation Therapy Alone. XRT given at a dose of 300 cGy per day on three consecutive days beginning 48 h after placement of an empty CPP:SA polymer was well tolerated by mice, with no mortality or significant morbidity. Histological examination of the brains of these animals more than 100 days after completion of XRT revealed a small amount of hippocampal and Purkinje cell neuronal injury. A trace amount of white matter change and cerebral gliosis were also noted. Histological changes seen following XRT alone were less pronounced than those seen with local chemotherapy or with local chemotherapy plus XRT.

BCNU Dose Escalation for Toxicity. BCNU alone was well tolerated by mice at doses up to 20% loading of polymer by weight (Table 3): 80% of the animals survived 100 days following local therapy with 20% BCNU-loaded polymer alone. With concurrent radiation therapy, 20% BCNU polymers were toxic: two of five animals died during the first week and only two survived to 100 days. However, 10% loaded BCNU polymer was well tolerated with subsequent radiation therapy. Histology of the animals receiving 10% loaded BCNU polymer and radiation therapy revealed mild to moderate neuronal dropout in the hippocampus and Purkinje cell layer of the cerebellum. Gliosis and white matter changes were also mild, but the histological findings were slightly more extensive than with XRT alone. Doses of 20% loading by weight for BCNU polymer alone and 10% loading by weight for BCNU polymer with radiotherapy were selected for efficacy trials.

Dose Escalation for Carboplatin Toxicity. Analogous dose-escalating toxicity trials for carboplatin with and without concurrent radiation therapy were performed (Table 4). Without radiation therapy, carboplatin was well tolerated at 1% loading but caused significant toxicity at 3% loading. Histology of the brains of mice receiving 1% loading of carboplatin without radiation showed mild to moderate neuronal dropout and gliosis. White matter changes were mild. Mice receiving 3% loaded polymer had significant neuronal dropout in the hippocampus and in the Purkinje cell layer of the cerebellum, as well as pronounced gliosis. This correlated well with the survival data, as 4 of 5 animals treated at this higher dose died within the first 8 days. With addition of radiation therapy, 2 of 5 animals receiving 1% loading died during the first week after implant. In contrast, 0.5% loaded carboplatin polymer and subsequent XRT was well tolerated. Histological changes in animals receiving 0.5% loaded carboplatin polymer with XRT were similar to those seen following administration of 1% carboplatin polymer without XRT and to those seen following administration of 10% loaded BCNU polymer with XRT. Therefore, 1 and 0.5% loadings of carboplatin without and with radiation, respectively, were selected for efficacy trials.

Dose Escalation for Camptothecin Toxicity. The dose escalation trials of camptothecin with and without radiation demonstrated that 10% loaded camptothecin polymers were well tolerated without or

Table 3 *Dose escalation trial of BCNU-loaded CPP:SA polymer (5-mg size) in mice without and with concurrent radiation therapy*

Use of radiation therapy[a]	Dose of BCNU (% loading by weight of a 5-mg polymer)	No. of animals surviving 100 days[b]	Death dates following polymer placement
Without concurrent radiation therapy	0	5/5	
	5	5/5	
	10	5/5	
	15	5/5	
	20	4/5	13
With concurrent radiation therapy	0	5/5	
	1	5/5	
	5	5/5	
	10	4/5	88
	20	2/5	4, 7, 72

[a] XRT given as a single fraction of 300 cGy/day for three successive days beginning 2 days after polymer placement.

[b] expressed as number of mice surviving 100 days/number of mice receiving polymer implant.

Table 4 *Dose escalation trial of carboplatin-loaded CPP:SA polymer (5-mg size) in mice without and with concurrent radiation therapy*

Use of radiation therapy[a]	Dose of carboplatin (% loading by weight of a 5-mg polymer)	No. of animals surviving 100 days[b]	Death dates following polymer placement
Without concurrent radiation therapy	0.5	5/5	
	1	5/5	
	3	1/5	5, 6, 8 (2 mice died on day 8)
With concurrent radiation therapy	0	5/5	
	0.5	5/5	
	1	3/5	2, 7

[a] XRT given as a single fraction of 300 cGy/day for three successive days beginning 2 days after polymer placement.

[b] expressed as number of mice surviving 100 days/number of mice receiving polymer implant.

Table 5 *Dose escalation trial of camptothecin-loaded CPP:SA polymer (5-mg size) in mice without and with concurrent radiation therapy*

Use of radiation therapy[a]	Dose of camptothecin (% loading by weight of a 5-mg polymer)	No. of animals surviving 100 days[b]	Death dates following polymer placement
Without concurrent radiation therapy	2	6/6	
	5	6/6	
	10	6/6	
With concurrent radiation therapy	1	5/5	
	3	5/5	
	6	5/5	
	10	4/5	38

[a] XRT given as a single fraction of 300 cGy/day for three successive days beginning 2 days after polymer placement.
[b] expressed as number of mice surviving 100 days/number of mice receiving polymer implant.

with radiation therapy (Table 5). Prior experiments in these murine models have shown that higher intracranial doses of camptothecin are toxic (data not shown). Histological examination of the animals receiving camptothecin revealed mild to moderate neuronal dropout, gliosis, and white matter changes. The changes were more extensive when radiation was also given. In general, the histological changes following camptothecin administration were more pronounced than with either BCNU or carboplatin.

Efficacy of BCNU-loaded Polymer. BCNU-loaded polymer given in conjunction with radiation therapy was effective against all four metastatic tumor lines (Table 6). In treating intracranial B16-F10 melanoma (Fig. 1*A*), BCNU polymer with subsequent radiation therapy was the most effective (median survival, 35 days; IQR, 31–36.5 days; $P = 0.0005$); survival of the animals receiving combination therapy was significantly prolonged in comparison with controls, which received therapy with blank polymer (median survival, 21.5 days; IQR, 20–23 days; $P = 0.0005$). BCNU polymer alone (no XRT) prolonged survival significantly (median survival, 26 days; IQR 25.5–30 days; $P = 0.001$). Radiation therapy alone was also effective (median survival, 28 days; IQR, 28–28 days; $P = 0.0005$). The combination of BCNU and XRT was significantly more effective than either BCNU or radiation alone ($P \leq 0.006$).

In treating solitary RENCA lesions in the brain (Fig. 1*B*), the combination of BCNU polymer and radiation therapy (median survival, 38.5 days; IQR, 35–42 days) was significantly more effective than BCNU polymer alone (median survival, 13 days; IQR, 13–27 days), radiation alone (median survival, 15 days; IQR, 14–17.5 days), or blank polymer (median survival, 12 days; IQR, 12–13.5 days). Both BCNU alone and radiation alone prolonged survival modestly but significantly in comparison with empty polymer ($P \leq 0.005$).

CT26 colon carcinoma (Fig. 1*C*) was effectively treated by BCNU-polymer and radiation therapy (median survival, 38.5 days; IQR, 13–68 days; $P < 0.001$ *versus* control); animals treated by radiation alone (median survival, 44 days; IQR, 33 to >80 days; $P < 0.001$ *versus* control), and with BCNU treatment without XRT also had a prolonged survival (median survival, 37 days; IQR, 13–68 days), although BCNU treatment alone did not reach statistical significance in comparison to controls (median survival, 23.5 days; IQR, 20–29 days). There was no statistical difference between the BCNU polymer plus radiation, BCNU polymer alone, and radiation alone groups.

The Lewis lung carcinoma intracranial tumors were relatively refractory to all three therapies tested (Fig. 1*D*). Only the combination of BCNU polymer and radiation afforded a small but statistically significant survival advantage (polymer plus radiation: median survival, 23 days; IQR, 22–26 days; blank polymer: median survival, 21 days; IQR, 20–22 days; $P = 0.001$). Of note, the IQRs of the two groups did not overlap.

In general, autopsies revealed that animals treated with either polymer, XRT, or combination therapy (polymer and XRT) died as a result either of local tumor mass at the injection site or as a result of diffuse intracranial spread of the disease. Spread of tumor outside the CNS was not seen. Untreated control animals uniformly had large local tumors at the injection site. In addition, many had evidence of spread of their neoplastic disease throughout the CNS. These findings were consistent for all four tumor lines tested.

Efficacy of Carboplatin-loaded Polymer. Carboplatin given in conjunction with radiation therapy prolonged survival of animals with intracranial CT26 colon adenocarcinoma in comparison with control animals (median survival, 33 days; IQR, 22.5–33 days; *versus* median survival, 20.5 days; IQR, 18–27 days, respectively; $P = 0.013$). This combination also prolonged survival of animals with intracranial RENCA (median survival, 15 days; IQR, 13.5–15.5 days; *versus* controls: median survival, 12 days; IQR, 12–13.5; $P < 0.01$; Table 7). Carboplatin polymer alone prolonged survival in animals with intracranial B16 melanoma (median survival, 27 days; IQR, 19–27 days; *versus* controls: median survival, 16.5 days; IQR, 10–20 days; $P = 0.022$), although the combination of radiation and the lower dose polymer (0.5% given with radiation, 1.0% given without radiation) did not prolong survival in the B16 model.

Efficacy of Camptothecin-loaded Polymer. Camptothecin polymer given in conjunction with radiation therapy prolonged survival only in mice in the melanoma model (median survival, 27.5 days; IQR, 14–29 days; *versus* controls: median survival, 19 days; IQR, 16–19 days; $P = 0.043$; Table 8). Camptothecin showed no effect on the other tumor lines either alone or with concurrent XRT.

Efficacy of Radiation Therapy Alone. A pooled analysis of the groups receiving XRT only from the multiple drug efficacy trials was done for each tumor line to evaluate the effectiveness of XRT as monotherapy in these models. Radiation therapy was effective against B16 melanoma (radiation group: $n = 27$; median survival, 27 days; control group: $n = 27$; median survival, 19 days; $P = 0.001$) and against CT 26 colon carcinoma intracranially (radiation group: $n = 27$; median survival, 27 days; control group: $n = 31$; median survival, 22 days; $P = 0.013$). For the RENCA model, a modest prolongation was seen (radiation group: $n = 13$; median survival, 15 days; control group: $n = 15$; median survival, 12 days; $P = 0.001$). For the Lewis lung model, the control group ($n = 30$; median survival, 21.5 days) was not statistically different from the radiation group ($n = 29$; median survival, 23 days).

Table 6 *Median survivals of mice treated with polymer-delivered BCNU, radiation therapy, or both in four separate models of solitary brain metastasis*

Intracranial tumor model	Treatment[a]			
	Blank polymer	XRT alone	20% loaded BCNU polymer alone	10% loaded BCNU polymer and XRT
B16 melanoma	21.5 (20–23)	28[b] (28–28)	26[b] (25.5–30)	35[b] (31–36.5)
RENCA	12 (12–13.5)	15[b] (14–17.5)	13[b] (13–27)	38.5[b] (35–42)
CT26 colon adenocarcinoma	23.5 (18–27)	44[b] (25–36.5)	37 (19–55.5)	38.5[b] (22.5–35)
Lewis lung carcinoma	21 (20–22)	21 (20–22)	21.5 (14.5–24.5)	23[c] (22–26)

[a] Median survival in days is presented with IQR (25–75% survival) in parentheses below it.
[b] $P < 0.005$.
[c] $P \leq 0.05$.

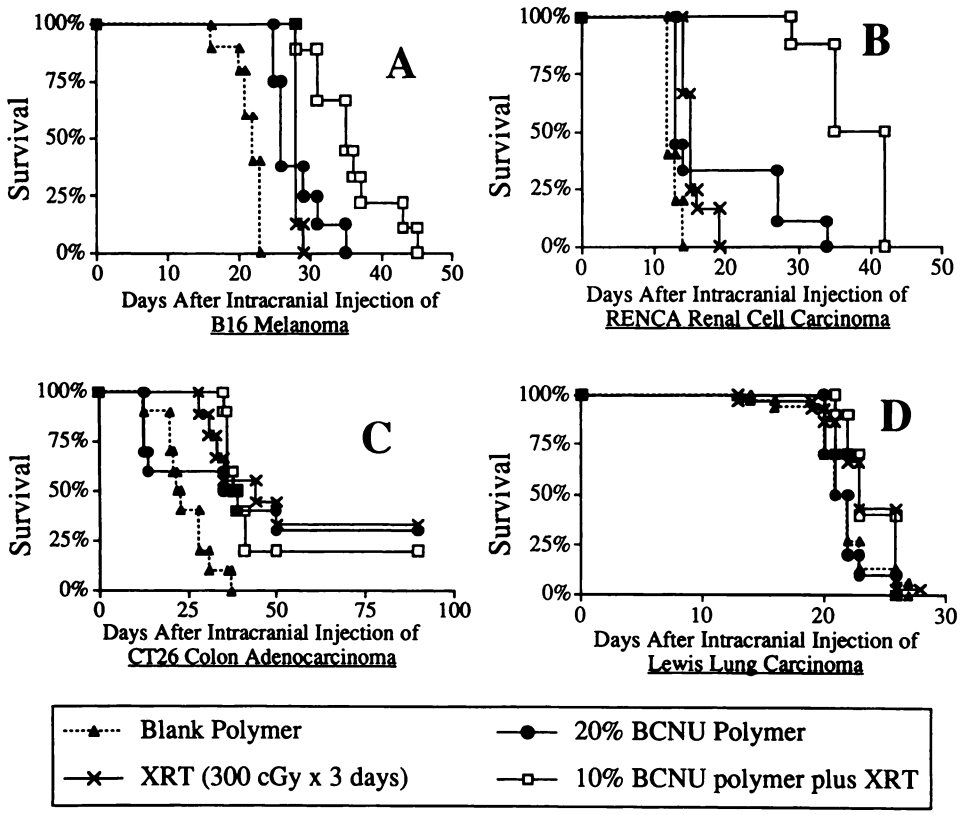

Fig. 1. The efficacy of BCNU, delivered locally by biodegradable polymer, in treating four models of solitary intracranial brain metastasis is demonstrated. Kaplan-Meier survival curves demonstrate survival following intracranial injection of B16-F10 melanoma (*A*), RENCA (*B*), CT26 colon adenocarcinoma (*C*), and Lewis lung carcinoma (*D*). Four treatment groups are included for each tumor: blank polymer control (▲), 20% loaded BCNU polymer placed in tumor bed (●), radiation therapy with 300 cGy per day for 3 days (×), and 10% loaded BCNU polymer followed by radiation therapy with 300 cGy per day for 3 days (□). *A*, BCNU polymer, radiation therapy, and combination therapy with BCNU and XRT all prolonged survival in the intracranial B16 model as compared with control ($P < 0.001$). The combination therapy was superior to either BCNU alone or XRT alone ($P < 0.006$). *B*, BCNU polymer, radiation therapy, and combination therapy with BCNU and XRT all prolonged survival in the RENCA intracranial model as compared with control ($P < 0.007$). The combination therapy was superior to either therapy (BCNU alone or XRT alone; $P < 0.001$). *C*, radiation alone and radiation plus 10% loaded BCNU polymer were superior to blank polymer in the CT26 colon adenocarcinoma intracranial model. The polymer alone (20%), radiation alone, and the polymer plus radiation groups were not statistically different. *D*, the combination of BCNU and XRT prolonged survival by 2 days (median, 21 days in blank group, 23 days in combination group), a difference that reached statistical significance ($P = 0.001$) in the Lewis lung carcinoma intracranial model. Of note, the IQR survival of the blank group (20–22 days) did not overlap the IQR of survival of the combined therapy group (22–26 days).

Table 7 *Median survivals of mice treated with polymer-delivered carboplatin, radiation therapy, or both in four separate models of solitary brain metastasis*

	Treatment[a]			
Intracranial tumor model	Blank polymer	XRT alone	1% loaded carboplatin polymer alone	0.5% loaded carboplatin polymer and XRT
B16 melanoma	19 (16–19)	16 (14–24)	27[b] (19–27)	16.5 (10–20)
RENCA	12 (12–13.5)	15[c] (14–17.5)	13 (12–14)	15[c] (13.5–15.5)
CT26 colon adencarcinoma	20.5 (18–27)	28 (25–36.5)	33[d] (19–55.5)	33[c] (22.5–35)
Lewis lung carcinoma	22 (20–24.5)	23 (22–26)	19 (16.5–21)	23 (20.5–26)

[a] Median survival in days is presented with IQR (25–75% survival) in parentheses below it.
[b] $P \leq 0.05$.
[c] $P \leq 0.01$.
[d] $P = 0.064$.

DISCUSSION

We have demonstrated that a new modality for treating solitary metastatic brain tumors, controlled delivery of chemotherapy from biodegradable polymers, significantly prolongs survival in murine models of this disease. With minimal morbidity, this new approach can be combined with the present therapy, surgical resection, and XRT. In three of four brain metastases models, this combination of surgically implanted local chemotherapy and radiation was superior to radiation alone in prolonging survival. Of the three agents tested, BCNU was the most effective.

Surgically implanted, chemotherapy-laden biodegradable polymers have been used both clinically (24–26) and in experimental models (27, 29, 33–35) to treat primary malignant brain tumors. The polymers were

Table 8 *Median survivals of mice treated with polymer-delivered camptothecin, radiation therapy, or both in four separate models of solitary brain metastasis*

	Treatment[a]			
Intracranial tumor model	Blank polymer	XRT alone	10% loaded camptothecin polymer alone	10% loaded camptothecin polymer and XRT
B16 melanoma	19 (16–19)	24[b] (22.5–27)	20 (8–21.5)	27.5[c] (14–29)
RENCA	12 (12–13.5)	15[d] (14–17.5)	13 (12–14.5)	13 (13–13)
CT26 colon adenocarcinoma	22 (21–26)	22.5 (17.5–26)	20.5 (17–23)	22 (17–24.5)
Lewis lung carcinoma	22 (21.5–23)	26[c] (23–33)	23 (21–26)	24.5 (21–26)

[a] Median survival in days is presented with IQR (25–75% survival) in parentheses below it.
[b] $P < 0.005$.
[c] $P \leq 0.05$.
[d] $P \leq 0.01$.

initially developed for therapy of gliomas because local control of tumor growth was considered to be important in treating primary brain tumors (27). Local control of metastatic tumors is also important because of the high morbidity and mortality of CNS metastases (3, 13). Furthermore, because the CNS is becoming a more common site for recurrence following effective systemic therapy (10–12), control of disease in the brain is an increasingly important factor in determining patient survival.

To study the role of chemotherapy-laden biodegradable polymers in treating brain metastases, three chemotherapeutic agents were selected. Each was chosen because it possessed unique properties that make it particularly suitable for local delivery in the CNS. BCNU is an alkylating agent that is lipid soluble. Like other nitrosoureas, BCNU has an almost infinite number of potential sites of action in the cell, so that it does not exhibit a plateau in antitumor effect with increasing doses (36). Therefore, the high drug levels achieved by local delivery (28) may provide improved efficacy over the lower levels obtainable by systemic delivery to the brain. In addition, BCNU polymer implants have been recently cleared by the United States Food and Drug Administration for clinical use in glioma patients. Therefore, clinical trials for treatment of metastatic brain tumors with BCNU-loaded polymer are readily possible. Carboplatin was chosen because it is highly water soluble and therefore may diffuse farther from the polymer than more lipophilic agents. In addition, it has been difficult to achieve adequate brain concentrations of carboplatin via systemic administration because of its water solubility. For these two reasons, carboplatin is an excellent candidate for local delivery with polymers. Camptothecin was selected because its mechanism of action, inhibition of the enzyme topoisomerase I, is different from either BCNU or carboplatin. Therefore, tumors that are resistant to alkylating agents could still be susceptible to camptothecin. Camptothecin, although quite effective in killing tumor cells, has been limited in its clinical utility due to systemic toxicity. Therefore, camptothecin is also an ideal candidate for local brain delivery. In fact, polymer-delivered camptothecin significantly prolongs survival in a rat 9L glioma model (33).

The tumors studied in these experiments were chosen because they are common histopathological types that spread to the brain and because they are traditionally radioresistant, increasing the need for effective chemotherapy or radiosensitization in their treatment. The combination of lung, colon, and renal cell carcinomas and melanoma represents a majority of brain tumor metastases seen.

Using these three agents and the four secondary brain tumor models, the role of chemotherapy-laden biodegradable polymers in treating brain metastases was defined using the three experimental groups (groups 2–4) and one control group (group 1). These groups were designed to model either future strategies, which include locally delivered chemotherapy with or without subsequent radiation therapy (groups 3 and 4), or the current standard therapy, surgery to remove macroscopic disease followed by whole brain XRT to treat microscopic tumor foci (group 2). Animals in group 2 received stereotactic injections of tumor cells and 7 days later began radiotherapy. At the time these animals began radiation therapy, their tumor burden was similar to that of a patient who has undergone surgical resection of a brain metastasis and also is starting radiation therapy. Typically, such a patient has only microscopic foci of residual tumor because most metastatic lesions are well circumscribed, and thus, complete macroscopic resection can be achieved. Similarly, when the mouse brain was examined 5–7 days after tumor cell inoculation, only microscopic tumor deposits can be seen. For this reason, we believe the treatment paradigm for group 2 is a good model of current standard therapy. In addition, because there was no macroscopic disease present in the mice as they began XRT, no surgical resection was attempted. We have previously seen that efforts to resect these murine tumors at day 5 have no effect on survival.

We envisioned two clinical situations in which polymer-based chemotherapy may be particularly useful in treating brain metastases. First, patients with initial presentations of brain metastasis could undergo surgery to remove macroscopic disease, have chemotherapy-laden polymer placed in the resection cavity, and finally have external beam radiation therapy. This scenario is modeled by group 4 in our studies. Mice in group 4 received stereotactic injection of tumor on day 0, surgery for polymer placement on day 5, and XRT on days 7–9. A second group of patients in whom polymer-based chemotherapy may be of use is those who have had recurrence of a brain metastasis or development of a second brain tumor metastasis at a separate site *after* they have received a full course of radiation therapy. These patients are not candidates for further conventional radiation, and they are modeled by our group 3 (drug-loaded polymer placed on day 5, no XRT). Of note, these patients may tolerate a higher dose of local chemotherapy because the possible additive toxicity between chemotherapy and radiation therapy will be avoided.

In these models, BCNU was the most effective agent tested. For three of the four tumor models studied, a combination of local BCNU and radiation afforded longer survival than radiation alone. Only in the colon carcinoma model, when all three therapies (BCNU, BCNU and XRT, and XRT) prolonged survival, did the efficacy of the combination of XRT and chemotherapy not exceed XRT alone. BCNU-loaded polymers, therefore, can be safely given before XRT in these models and afford a survival advantage when administered in this way. In previous clinical trials in glioma patients, polymer wafers were safely placed in a surgical resection cavity with minimal morbidity, were well tolerated when given prior to XRT, and prolonged survival (24–27). Given the efficacy findings in these models and the clinical evidence that this therapy can be safely administered, proceeding to a clinical trial evaluating the role of locally delivered BCNU in treating brain metastases is reasonable.

In addition to efficacy, we also examined toxicity to determine the maximum tolerated doses for each locally delivered agent. Of note for BCNU and carboplatin, a higher dose was tolerated when no XRT was given. This suggests that locally delivered BCNU or carboplatin can act as a radiosensitizing agent. Although a lower dose of BCNU or carboplatin polymer must be used when XRT is also given in order to protect the brain, rapidly dividing tumor cells may be much more sensitive to the additive effects of chemotherapy and subsequent radiation therapy than are nondividing neurons. This hypothesis is supported by the finding that BCNU and XRT was more effective than BCNU alone in three of the four models tested and equally effective in the fourth. In addition, systemically administered BCNU or cisplatin, which is closely related to carboplatin, have been shown to potentiate the efficacy of cranial irradiation in primary glioma models in rodents (37).

Lung carcinoma, the most common source of brain metastasis, was the least sensitive experimental tumor tested. There is, however, clinical evidence that lung carcinomas may respond to local chemotherapy despite the relative resistance to therapy seen with Lewis lung carcinoma in our model. A recent study of patients with lung metastases to the brain treated systemically with a nitrosourea (fotemustine) and a platinum-containing alkylating agent (cisplatin) showed a 28% response to systemic therapy. However, hematological toxicity was high and dose-limiting (38). In the same patients, polymers could be used to deliver large doses of either of two similar agents, BCNU and carboplatin, directly at the tumor site, achieving higher levels than are possible with i.v. therapy and avoid dose-limiting systemic toxicity. In addition, multi-agent local chemotherapy for brain metastases may further improve efficacy. Finally, lung cancer metastases represent a diverse group of

histopathologies, including squamous cell carcinoma, adenocarcinoma, and large cell undifferentiated carcinoma. We would expect that the response of these different histopathological variants and perhaps even the response of individual patients who share similar histological diagnoses to locally delivered chemotherapy will be heterogeneous.

The biologically equivalent dose (39) of the radiation regimen used in this study is 30% of the biologically equivalent dose of the standard regimen used for brain metastases in humans (30 Gy/treatment for 10 treatments). This dosage was selected for two reasons. First, it was well tolerated when given to mice in conjunction with polymer-delivered local chemotherapy. Second, the dose provides a modest protective effective in several but not all of the models of intracranial metastasis studied, approximating the response seen in patients treated with standard XRT.

In conclusion, combined intracranial delivery of chemotherapy from biodegradable polymers and XRT controls the growth of solitary brain metastases in experimental models. In three of four tumor models, the greatest prolongation in survival was achieved by a combination of polymer-delivered chemotherapy and radiotherapy, and the most effective agent tested was BCNU.

Based upon the efficacy of BCNU-loaded polymer and radiation therapy demonstrated here and upon prior clinical studies that demonstrated safety, a clinical trial of BCNU-loaded polymer as an adjuvant therapy during the resection of solitary metastatic lesions can be proposed. As other agents become available for clinical use via polymer delivery, these data suggest they too will be useful additions to the armamentarium available for the treatment of brain metastases.

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