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Optimizing interstitial delivery of BCNU from controlled release polymers for the treatment of brain tumors

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Abstract Two approaches for improving the interstitial administration of carmustine (BCNU) using 3.8% loaded poly(carboxyphenoxypropane-sebacic acid), an implantable biodegradable anhydride which significantly prolongs survival in patients with recurrent malignant gliomas, were evaluated. First, increasing the ratio of carboxyphenoxypropane (CPP) to sebacic acid (SA) in the polymer increases its hydrolytic stability, thus prolonging its half-life in vivo, and extending the period of drug release. A second approach is to increase the dose of drug loaded into the polymer. This study evaluated the relative merits of these two approaches by comparing release kinetics, safety, and efficacy of escalating BCNU doses in polymers with 20:80 and 50:50 ratios of CPP to SA.

At the highest dose tested, the 50:50 polymer released BCNU 2.5 times as long in vitro as the 20:80 polymer. Both formulations were nontoxic in rat brains for all BCNU doses tested except 32%. The 20:80 and 50:50 polymers were equally effective in the rat intracranial 9L-glioma model. A dose-response relationship for BCNU was observed (hazard ratio 0.8354 for each mg/kg increase, $P < 0.001$). The two highest loading doses of BCNU improved survival 40-fold ($P < 0.001$). The 20% BCNU-loaded 20:80 polymer achieved the best balance of toxicity and antitumor efficacy, yielding a 75% long-term survival rate. Further evaluation of this polymer in monkeys suggests that it might be used with acceptable toxicity. This study establishes that a dose-escalation strategy for improving BCNU controlled-release polymers is more effective than adjusting the ratio of CPP to SA to prolong drug release.

Key words BCNU · Drug delivery · Brain tumors · Gliomas

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Introduction

Carmustine (BCNU) is the most commonly used and most effective chemotherapeutic agent for brain tumors. Systemic administration of this drug has provided modest improvements in patient survival, but its efficacy has been limited by myelosuppression, hepatic toxicity, and pulmonary fibrosis [12]. Moreover, despite therapy, the local recurrence rate of primary brain tumors remains high [11]. In order to minimize systemic toxicity while maximizing local drug concentrations within primary brain tumors, interstitial BCNU therapy with implantable biodegradable polymers has been developed and tested for use against malignant gliomas in a series of preclinical studies, which showed that the polymers are biocompatible, safe, and effective in experimental glioma models [1, 3, 6, 10, 15–19]. These preclinical findings led to phase I–III clinical trials which demonstrated that 3.8% BCNU (by weight) incorporated into the drug delivery polymer is safe and effective for treatment of patients with recurrent malignant gliomas [2, 4, 5]. Although the clinical benefits achieved with this polymer formulation are significant, improvement in survival is modest [5]. We therefore designed this series of experiments to explore whether modifications in the BCNU polymer formulation could improve antitumor efficacy without unacceptable toxicity.

The controlled-release biodegradable polymer used in these studies was carboxyphenoxypropane copolymerized with sebacic acid [p(CPP:SA)]. Two strategies

for improving the polymer formulation were considered and tested. First, by increasing the ratio of CPP to SA in the polymer, it is possible to increase its hydrolytic stability, thus prolonging its half-life in vivo and extending the period of drug release from the polymer. Second, by simply increasing the amount of drug loaded into the polymer, it may be possible to enhance local drug delivery and prolong survival. These experiments were designed to assess the relative merits of these two alternative approaches by comparing in vitro kinetics of drug release as well as in vivo safety and efficacy of escalating BCNU doses in polymers consisting of 20:80 and 50:50 ratios of CPP to SA.

Materials and methods

Polymer fabrication

The p(CPP:SA) polymers were obtained from Avi Domb (Hebrew University, Jerusalem, Israel) in 20:80 and 50:50 formulations. In order to refine the polymers before use, they were dissolved in dichloromethane (10% w/v), then immediately precipitated by adding them slowly to a stirred solution of diethyl ether and hexane (1:1 v/v). The precipitated polymer was then isolated by filtration, dried in a vacuum desiccator, packed under dry nitrogen, and stored in a freezer at $-30\,^{\circ}C$ until use.

BCNU was incorporated into the refined polymers according to the method of Domb and Langer [9] to yield polymers containing 0, 4, 8, 12, 20, or 32% BCNU by weight in both 20:80 and 50:50 p(CPP:SA) formulations. This dose-escalation sequence is a Fibonacci series based upon the dose previously studied in human phase I–III clinical trials (3.8% BCNU by weight) [2, 5]. The BCNU-polymer mixtures were dissolved in dichloromethane to yield 10% solutions (w/v). The solvent was then evaporated in a vacuum desiccator. The resulting dried polymers were compression-molded into discs weighing approximately 10 to 12 mg using a stainless steel mold with internal diameter of 3.0 mm in a Carver Press (No. 400, Littlestown Hardware and Foundry Co., Littlestown, Pa.) at approximately 200 p.s.i. The individual weights of all polymer discs were recorded.

Assays of BCNU release from polymers

Polymer discs of 20:80 and 50:50 p(CPP:SA) formulations with 4% and 32% BCNU by weight were assayed for BCNU release. Individual discs were placed in 1.5-ml vials containing 1.0 ml normal saline. The vials were capped to prevent evaporative loss and placed in an incubator at $37\,^{\circ}C$. The medium was replaced at specified times during the 3-week incubation period, and the recovered solution was assayed for the presence of BCNU by high-pressure liquid chromatography (HPLC). These analyses were performed with a Beckman System Gold (consisting of Autosampler 507, Programmable Solvent Module 126AA, and Programmable Detector Module 166 from Beckman Instruments, San Ramon, Calif.) controlled and integrated by a personal computer (Dell System 200, Dell Computer Corporation, Austin, Tx.) and equipped with a 3.9×300 mm μBondapack C18 column (Waters Associates, Milford, Mass.). The flow rate was 1 ml/min, the mobile phase consisted of methanol/water (40:60), and UV detection was performed at a wavelength of 237 nm. Under these conditions, the retention time of BCNU was 5.8 min.

Animals

A total of 192 male Fischer 344 rats weighing 200 to 250 g were obtained from Harlan Sprague Dawley (Indianapolis, Ind.) for the toxicity and efficacy experiments. All rats were housed in standard facilities with not more than four rats per cage and were given free access to water and ProLab RMH 1000 Formula (Agway, Syracuse, N.Y.).

Five adult male cynomolgus monkeys (*Macaca fascicularis*) weighing from 4 to 6 kg, obtained from White Sands Research Center, New Mexico, were quarantined for 6 weeks until found to be in good health by the veterinary staff, then were transferred to standard primate housing facilities. All monkeys were caged individually under veterinary care and supervision and were given free access to water and ProLab Primate 18 Formula (Agway) supplemented with fresh fruit.

Anesthesia

Rats were anesthetized with intraperitoneal injections of 3 to 4 ml/kg of a stock solution of normal saline containing ketamine hydrochloride (25 mg/ml), xylazine (2.5 mg/ml), and ethyl alcohol (14.25%).

Monkeys were anesthetized after a 12-h fast (without restricting water consumption) with an intramuscular injection of ketamine hydrochloride (10 mg/kg), then transferred to the operating suite. A catheter was inserted into a calf vein for continuous intravenous infusion of Ringer's lactate solution throughout the procedure. For the craniotomy, the monkeys were intubated endotracheally under direct vision and maintained on halothane inhalational anesthesia with continuous cardiac and respiratory monitoring. During the MRI study, anesthesia was achieved with an intravenous bolus of ketamine hydrochloride (10 mg/kg) supplemented with half this dose midway through the study. After anesthesia, the monkeys were returned to their cages and were observed closely until they awakened.

Toxicity studies

Rats

The scalps of 96 anesthetized rats were shaved and disinfected. These animals were randomly assigned to treatment groups receiving 20:80 or 50:50 p(CPP:SA) polymers containing 0, 4, 8, 12, 20, or 32% BCNU. Each animal was then weighed. A polymer disc was inserted into the brain parenchyma in the left parietal region of each animal using microsurgical techniques described previously [16].

The animals were then checked daily for physical or behavioral evidence of toxicity, such as decreased alertness, impaired grooming, lacrimal debris around the eyes, focal motor deficits, or gait disturbances. Autopsies were performed on the few animals that died before the end of the experiment. All remaining animals were euthanized after 200 days of observation and autopsies were performed. Survival data, autopsy findings, histologic findings, and changes in body weight were recorded.

Monkeys

After induction of anesthesia and placement of an intravenous catheter, cardiac monitor, and esophageal stethoscope, each monkey was positioned prone. The scalp over the operative site was shaved, disinfected, and draped in sterile fashion. A left frontal skin incision was made and scalp bleeding was controlled with electrocautery. The underlying temporalis fascia and muscle were then divided with

electrocautery. A high speed air drill, a mastoid rongeur, and a curette were then used to create a 1.0×1.5 cm ovoid craniectomy in the left frontal bone extending posteriorly from the supraorbital ridge. The dura was incised and a small linear corticotomy was made by using a combination of bipolar cautery and gentle suction. Once hemostasis had been achieved, a single sterile 200-mg polymer wafer (20:80 p(CPP:SA) with 20% BCNU by weight was inserted into the cortical defect until it was completely beneath the cortical surface. The sterile field was irrigated and a rectangular piece of Gelfoam was placed to fit the craniectomy defect. The temporalis muscle fascia was closed with a running 3.0 vicryl suture and the scalp was closed with interrupted 3.0 prolene sutures. The anesthetic was discontinued and each of the animals was extubated uneventfully. Once the monitors were disconnected and the intravenous catheter removed, the animals were transported back to the housing facility and observed while recovering from anesthesia.

A total of five monkeys underwent surgical implantation of the polymers containing BCNU. Two were sacrificed on the first postoperative day, one on the third, and one on the fifth postoperative day. Full autopsies were performed and the brains were examined histologically. The fifth animal was kept alive for long-term behavioral and radiographic observation. A magnetic resonance imaging (MRI) study, consisting of 3.0-mm precontrast axial T1 and T2 images followed by 3.0-mm postcontrast T1-weighted axial, coronal, and sagittal images, was performed 150 days after surgery. The contrast agent used for this study was 1.0 ml intravenous gadopentetate dimeglumine (Magnevist; Berlex Laboratories, Wayne, N.J.).

Tumor line

The 9L gliosarcoma, obtained in 1994 from the Brain Tumor Research Center, University of California, San Francisco, was maintained as solid subcutaneous masses in the flanks of male Fisher 344 rats. The tumor in carrier rats was passaged every 2 to 3 weeks.

Solid 9L gliosarcoma masses were removed in sterile fashion from the flanks of anesthetized carrier rats. The tumor was then cut into fragments measuring approximately 1 mm^3. The fragments were kept under sterile conditions in a covered petri dish with 0.9% saline until implantation into the brains of experimental animals or the flanks of new carrier rats.

Efficacy study

Tumor implantation

After exposure of the left parietal lobes of 96 anesthetized rats in the manner described above, the cortex and underlying white matter were aspirated with gentle suction until the dorsal brainstem was visualized. The resulting surgical defect was irrigated with sterile 0.9% saline until clear. Once the bleeding was controlled, a tumor fragment was placed in the cortical defect over the brainstem. The wound was closed with surgical clips. After awakening from anesthesia, the animals were returned to the animal housing facility.

Polymer implantation

On the fifth postoperative day, all animals were randomly assigned to treatment groups and anesthetized. The scalp was disinfected and surgical clips were removed. The skin incision was reopened, exposing the burr hole, and irrigated with sterile 0.9% saline. A polymer disc appropriate for the randomly assigned treatment was inserted through the burr hole into the tumor bed. The wound was again irrigated and closed with surgical clips. After awakening from anesthesia, animals were returned to the housing facility.

Euthanasia

Rats were euthanized with a 1.0 ml intraperitoneal injection of Euthanasia-6 Solution (Veterinary Laboratories, Lenexa, Kan.). The monkeys were first anesthetized with an intramuscular injection of 100 mg ketamine. An intravenous dose of 2.0 ml Euthanasia-6 Solution was then administered via a dorsal leg vein. Autopsies were performed immediately after euthanasia.

Statistical methods

The primary statistical outcome for the efficacy study was length of survival, without regard to the cause of death, from the time of tumor implantation. Event times were censored for rats still alive 200 days after tumor implantation. Differences in survival were expressed as hazard ratios. To control for the effect of more than one predictive factor simultaneously, such as polymer type and the weights of animals in each of the treatment groups, the proportional hazards regression model [8] was used.

Results

In vitro drug release

BCNU release into normal saline as a function of time was measured in triplicate for polymer discs with the 20:80 and the 50:50 p(CPP:SA) formulations containing 4% or 32% BCNU (by weight). There was no significant difference in the release profiles of the 20:80 and 50:50 polymer formulations at the lowest BCNU concentration. For each of the 4% BCNU-loaded polymers, a rapid initial burst of drug release within the first 24 h was followed by a 2- to 3-week period of slow release. For the 32% BCNU-loaded polymers, there was a similar initial burst of rapid drug release; however, the subsequent period of sustained slow release was 18 days for the 50:50 formulation, compared with 7 days for the 20:80 formulation. This represents an increase of more than 150% in the duration of detectable drug release for the 50:50 formulation at the highest BCNU concentration.

Intracranial toxicity in rats

Among the 96 animals in the rat intracranial toxicity experiment, there were 3 premature deaths; the remaining animals survived until day 200, when they were sacrificed and autopsied. One rat with a 12% BCNU-loaded 50:50 p(CPP:SA) polymer died on postoperative day 17. This animal had been gaining weight normally, and there was no behavioral evidence of toxicity. The autopsy findings were inconclusive and the cause of death was unknown. The two other animals that died before the conclusion of the experiment

were both from the 32% BCNU-loaded 20:80 p(CPP:SA) polymer treatment group. These rats both showed signs of systemic toxicity, suffering profound weight loss. They died 82 and 97 days after polymer implantation weighing only 69% and 59% of their initial body weight, respectively. Similarly, during this 200-day experiment, the other animals in this treatment group gained significantly less weight than did control animals treated with blank polymers. Although the 32% BCNU-loaded 20:80 polymer formulation appeared most toxic, animals treated with 32% BCNU-loaded 50:50 polymers also gained weight poorly (Fig. 1).

Upon histologic analysis, the brains were normal except for mild gliotic reactions around the surgical sites. The gliosis was most pronounced in brains from animals treated with 32% BCNU-loaded polymers. No signs of toxicity were evident in histologic specimens from the hearts, lungs, livers, or kidneys of these animals. The only significant pathologic findings were age-related myocardial [13] and renal [14] changes which occurred in all treatment groups, including the controls.

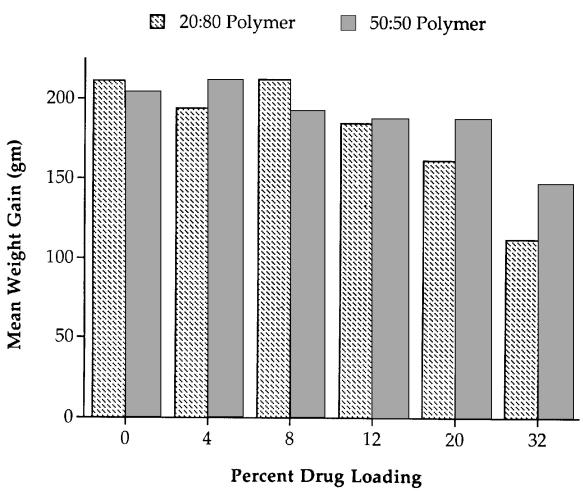

Fig. 1 Comparison of mean increases in body weight during the course of the rat toxicity study for animals treated with the 20:80 or 50:50 formulations of p(CPP:SA) containing different loading doses of BCNU ($n = 8$ for each treatment group)

Intracranial efficacy

Comparison of the 20:80 and 50:50 p(CPP:SA) showed no difference in survival for animals treated with these two different polymer formulations. There was, however, a dose-response relationship with improved survival in treatment groups receiving higher doses of BCNU (hazard ratio 0.8354 for each mg/kg dose increase, $P < 0.001$). Long-term survival, defined as greater than 120 days, was 75% in the 20% BCNU-loaded 20:80 p(CPP:SA) treatment group; 63% survived until the study was terminated at 200 days. These data are summarized in Table 1 and Fig. 2 and 3.

Analysis of histologic specimens confirmed the presence of large viable tumor masses at the original implantation sites in animals that died before the conclusion of this experiment. Small foci of necrosis totalling less than 10% of the tumor masses were usually present in these animals. In the long-term survivors, the tumor implantation sites appeared fibrotic with no viable neoplastic tissue. No evidence of toxicity was seen in the surrounding brains.

Intracranial toxicity in primates

One of the five monkeys with 20% BCNU-loaded polymer implants was observed to have intermittent periods of lethargy, somnolence, and two transient periods of unresponsiveness. No ictal motor activity was observed. This animal was sacrificed 5 days after surgery. At autopsy, the brain showed a small local

Table 1 Proportional hazards multiple regressions for intracranial efficacy study

Term	Hazard ratio	95% confidence bounds	*P*-value
Polymer weight (per g)	0.970	0.948–0.993	0.011
Dosage (mg/kg)	0.835	0.784–0.891	< 0.001
Polymer weight (per g)	0.973	0.951–0.995	0.018
Intermediate loading[a] vs placebo	0.089	0.041–0.192	< 0.001
High loading[b] vs placebo	0.025	0.010–0.063	< 0.001
4% BCNU vs placebo	0.080	0.033–0.196	< 0.001
8% BCNU vs placebo	0.132	0.057–0.301	< 0.001
12% BCNU vs placebo	0.095	0.039–0.230	< 0.001
20% BCNU vs placebo	0.039	0.015–0.102	< 0.001
32% BCNU vs placebo	0.027	0.010–0.074	< 0.001

[a] Intermediate loading 4, 8, and 12%
[b] High loading 20 and 32%

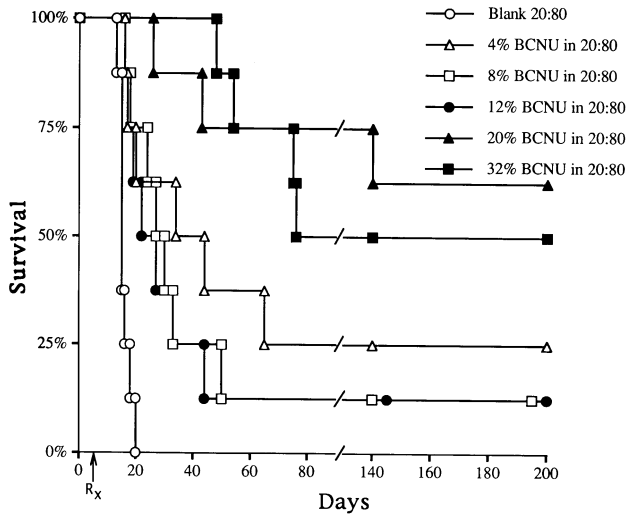

Fig. 2 Survival curves for animals bearing intracranial 9L-gliosarcomas treated 5 days after tumor implantation with polymers consisting of the 20:80 formulation of p(CPP:SA) containing escalating doses of BCNU compared with controls treated with empty (*blank*) polymers ($n = 8$ for each group)

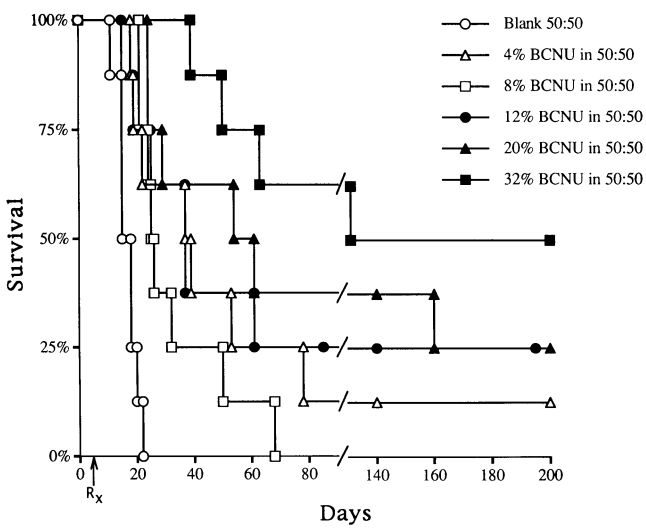

Fig. 3 Survival curves for animals bearing intracranial 9L-gliosarcomas treated 5 days after tumor implantation with polymers consisting of the 50:50 formulation of p(CPP:SA) containing escalating doses of BCNU compared with controls treated with empty (*blank*) polymers ($n = 8$ for each group)

hematoma at the surgical site, with no evidence of mass effect. The major abdominal and thoracic organs were grossly normal. Histologically, the brain showed the expected postoperative changes: mild to moderate edema, extravasated blood and mild inflammatory infiltrate in the surgical bed. Incidental findings in other organ systems included diffuse fatty change in the liver, mild multifocal bullous emphysema, and mild renal fibrosis with occasional tubular dilatation.

The other four monkeys recovered normally after surgery. There was no behavioral, systemic, or autopsy evidence of toxicity. Histologic examination of the brains showed changes the same as those described above.

An MRI study performed 150 days after polymer implantation in one of these monkeys showed no evidence of edema or mass effect. There was increased signal intensity on T1 images and decreased signal on T2 images in the operative site with minimal contrast enhancement within the surgical bed. The rest of the brain was radiographically normal (Fig. 4).

Discussion

The use of an implantable biodegradable polyanhydride polymer, p(CPP:SA), to deliver prolonged, high doses of BCNU directly into brain tumors, is a clinically effective method of drug administration that minimizes the toxicity of systemic drug exposure [2, 4, 5]. Given the increased survival observed clinically in patients with recurrent malignant gliomas treated with the 20:80 p(CPP:SA) polymer loaded with 3.8% BCNU by weight, we designed this series of laboratory studies to explore the relative merits of two possible approaches for improving the efficacy of this polymer system. These experiments established that increasing the loading dose of BCNU in the polymer was more effective than prolonging the period of drug release by adjusting the ratio of CPP and SA from 20:80 to 50:50.

The in vitro release kinetics study confirmed that, when loaded with 32% BCNU by weight, the 50:50 polymer formulation released chemically intact BCNU 150% longer than the 20:80 formulation. Since the HPLC assay used measured the concentration of intact BCNU in solution, and since BCNU has a short half-life in aqueous media at physiologic pH [7], it is likely that this study underestimated the amount and duration of drug release from the polymers. Nevertheless, this method was very useful for comparing the kinetics of drug release for different polymers.

Despite the more sustained drug delivery achieved with the 50:50 polymer, the in vivo dose-escalation study of efficacy demonstrated that there was no difference in survival for rats treated with the two p(CPP:SA) formulations. There was, however, a dose-response effect with increasing loading-doses of BCNU in both of the polymers. The 20% BCNU-loaded 20:80 polymer was most effective.

The slightly reduced efficacy of the 32% BCNU-loaded polymers may be explained by the results of the rat toxicity study. Both polymer/drug formulations were nontoxic at all loading doses tested except 32%. This dose caused systemic toxicity manifested by early deaths, impaired weight gain, and, histologically more

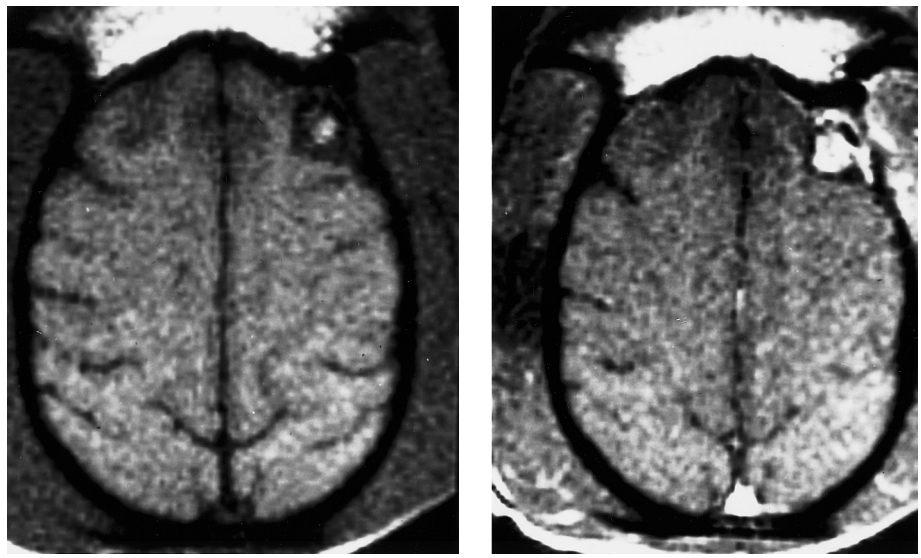

Fig. 4 Axial T1-weighted MRI scans through the surgical site of a monkey 150 days after polymer implantation within the left frontal lobe. The unenhanced scan (*left*) shows a focal area of increased signal within the surgical site. There is no edema or mass effect. After administration of 1.0 ml intravenous gadopentetate dimeglumine, enhancement is limited to the surgical site (*right*)

gliosis around the implant. Thus, the best balance of safety and efficacy in rats was 20% BCNU in the 20:80 formulation. This polymer was therefore further tested by intracranial implantation in monkeys.

When implanted in the brains of monkeys, the 20% BCNU-loaded polymer was generally very well tolerated. Brain edema and inflammatory infiltrates were seen locally around the polymer implantation site within 1 week of polymer implantation; however, there was no histologic evidence of toxicity in the brain, heart, lungs, liver, or kidneys. There was no radiographic evidence of toxicity on brain MRI scans 150 days after implantation.

These results demonstrate that it should be possible to improve the efficacy of local BCNU delivery safely with the same biodegradable polymer previously used clinically simply by increasing the loading dose of BCNU. A clinical dose-escalation trial is therefore planned.

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