

Interstitial Chemotherapy of the 9L Gliosarcoma: Controlled Release Polymers for Drug Delivery in the Brain¹

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ABSTRACT

The administration of drugs directly into the central nervous system using polymers as drug carriers may improve the treatment of malignant brain tumors. In this study, the effect of the interstitial, localized delivery of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) incorporated into controlled release polymers implanted adjacent to the 9L gliosarcoma was assessed in s.c. and intracranial (i.c.) models. In the s.c. experiment, the 9L gliosarcoma was implanted in the flank of rats and subsequently treated with BCNU either (a) delivered in controlled release polymers inserted adjacent to the tumor or (b) administered systemically by i.p. injections or by controlled release polymers inserted at a site distant from the tumor. The interstitial release of BCNU adjacent to the tumor in the flank resulted in a significant tumor growth delay of 16.3 days, as compared to a growth delay of 9.3 and 11.2 days obtained with the systemic administration of BCNU. In the i.c. experiment, the 9L gliosarcoma was implanted in the brain of Fischer 344 rats and treated either (a) with controlled release polymers containing BCNU inserted into the brain or (b) with the systemic i.p. administration of BCNU. The interstitial release of BCNU in the brain resulted in a significant 5.4- to 7.3-fold increased survival, compared with a 2.4-fold increased survival after the systemic administration of the same dose of BCNU. The two groups with i.c. tumors treated interstitially had 17 and 42% cures, but no long-term cures were obtained in the group treated with systemic therapy. The localized, controlled delivery of chemotherapeutic agents in the s.c. tissues and in the brain via polymeric carriers may be more effective than standard systemic chemotherapy. This approach could be used to deliver a wide variety of agents into the central nervous system to treat diverse neuropathological conditions which remain refractory to systemic therapy.

INTRODUCTION

Interstitial chemotherapy, the localized administration of drugs using polymer implants as vehicles for drug delivery, is an alternative to the systemic administration of chemotherapeutic agents for the treatment of malignant brain tumors. Theoretically, polymeric devices implanted i.c.³ could obviate the need for drugs to cross the blood-brain barrier and deliver drugs directly to the site of pathology, hence minimizing the systemic toxicity associated with current systemic therapy. Consequently, neoplastic cells could be exposed to unprecedented high regional drug levels over a prolonged period, and higher concentrations of drug at the site of tumor growth would be expected to improve effectiveness of any given chemotherapeutic regimen. In the present report, this hypothesis was tested by using controlled release polymers containing BCNU implanted in the flank and in the brain at the site of tumor growth to treat rats bearing the 9L gliosarcoma, a brain tumor syngeneic to the Fischer 344 rat (1-4). We have evaluated interstitial chemotherapy in terms of its effectiveness and toxicity as compared to standard systemic therapy.

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³ The abbreviations used are: i.c., intracranial; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; EVAc, ethylene-vinyl acetate copolymer; PCPP:SA, poly[bis(*p*-carboxyphenoxyl)propane-sebacic acid copolymer.

MATERIALS AND METHODS

Experimental Design

s.c. Study. Ninety rats underwent implantation of the 9L gliosarcoma in the flank. On the fifth day after implantation, all the animals were again operated for the initiation of treatment of the established tumor. Treatment consisted of: (a) systemic administration of BCNU (15 mg/kg) as a single intraperitoneal injection; (b) interstitial delivery of BCNU (15 mg/kg) in the tumor flank from a controlled release polymer inserted adjacent to the tumor; or (c) prolonged systemic administration of BCNU (15 mg/kg) from a controlled release polymer inserted in the flank contralateral to the tumor (Table 1). The systemic administration of BCNU in a single dose is the best reported treatment protocol for the 9L gliosarcoma in rats (5, 6). In the s.c. experiment a nonbiodegradable controlled release polymer, EVAc (7, 8), was used. EVAc is the prototype of the diffusion-regulated, nondegradable, controlled release polymer (9). The tumors were followed with serial measurements until they reached a volume of about 24 cm³, at which time the animals were sacrificed. The outcome of each treatment modality was evaluated by comparing the calculated time required for each tumor to reach 6000 mm³, a 10-fold increase in tumor volume from the time treatment was initiated.

i.c. Study. Ninety-six rats underwent a craniectomy. Sixty animals underwent implantation of the 9L gliosarcoma in the brain. The remaining 36 animals were assigned to three experimental groups to assess the toxicity of the i.c. and systemic administration of BCNU. The eight experimental groups are described in Table 2. On the fourth day after tumor implantation, all the animals were reoperated for initiation of treatment, which consisted of either systemic administration of BCNU (14 mg/kg) as a single i.p. injection or interstitial delivery of BCNU (14 mg/kg) in the brain via controlled release polymers implanted into the tumor. In the i.c. experiment two types of polymeric carriers were used: EVAc, as in the s.c. experiment, and the biodegradable polyanhydride PCPP:SA, an erosion-regulated controlled release polymer (10-12). The animals were examined twice daily for signs of chemotherapeutic toxicity or impending death. The long-term survivors were sacrificed 125 days after the initial operation. The effectiveness of each therapeutic intervention as well as the toxicity of either locally or systemically administered BCNU were assessed by comparing the survival of the animals in each group. Each animal underwent a full autopsy for determination of the cause of death.

Animals

Male Fischer 344 rats weighing about 200 g for the s.c. experiment and about 250 g for the i.c. experiment were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN), kept in standard animal facilities, four rats/cage, and given free access to Certified Rodent Chow No. 5002 (Ralston Purina Co., St. Louis, MO) and to Baltimore City water.

Anesthesia

The animals were anesthetized with an i.p. injection of 2-4 ml/kg of a stock solution containing ketamine hydrochloride (25 mg/ml), xylazine (2.5 mg/ml), and 14.25% ethyl alcohol in normal saline.

Tumor Line

The 9L gliosarcoma was obtained from Marvin Barker, Brain Tumor Research Center, University of California, San Francisco, CA. The cells were grown in Eagle's minimum essential medium with 10% fetal bovine serum, L-glutamine (398 µg/ml), penicillin (base; 80.5 units/ml), and streptomycin (80.5 µg/ml) (all products from GIBCO Laboratories, Grand Island, NY) in a humidified atmosphere of 5% CO₂ at 37°C. The cells were grown to confluence with medium changes every 2-3 days, detached with 0.25% trypsin in

Hanks' balanced salt solution without calcium and magnesium (GIBCO), and resuspended in medium. A solid tumor was established upon injection of the cell suspension in the flanks of male Fischer 344 rats. The cell line has been propagated as a solid tumor in the flank. To transfer the tumor, the flank of the carrier was shaved and prepared with 70% ethyl alcohol and povidone-iodine solution. Under sterile conditions, the tumor was excised, minced into pieces approximately 0.5 cm³, and dissociated into smaller fragments by pressing it through a 40 mesh (380 µm) screen in a Collector tissue sieve (Bellco Glass, Inc., Vineland, NJ). The tumor homogenate was suspended in Hanks' balanced salt solution without calcium or magnesium. The flank of the recipient was similarly prepared for the injection of the tumor homogenate. The tumors were passaged about every 2 weeks. The tumor sample used in this study had been passaged about 10 times at the time of implantation into the experimental animals.

Polymer Fabrication

EVAc (7) (40% vinyl acetate by weight; Elvax 40P) was obtained from the Du Pont Company, Wilmington, DE. The polymer was washed extensively in absolute ethyl alcohol, with total volume changes every 24 h, to extract inflammatory impurities from the EVAc (mainly the antioxidant butylhydroxytoluene). The presence of butylhydroxytoluene in the wash was monitored spectrophotometrically at 230 nm, and the washes were continued until the absorbance fell below 0.03 unit (8). The polymers were then dried in a vacuum desiccator over 4–5 days. BCNU was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, and incorporated into the EVAc matrix as described (13). Briefly, BCNU and EVAc in a 3:7 weight ratio were dissolved in methylene chloride (EVAc:methylene, 1:9 w/v). This BCNU-EVAc solution yielded dry polymers 30% loaded (w/w) with BCNU. The solution was poured into cylindrical glass molds at –70°C. The resulting polymer cylinders were then transferred to glass plates at –30°C and allowed to dry for about 7 days. The 30% BCNU-EVAc cylinders were then cut into 10- or 11.5-mg discs for s.c. or i.c. implantation, respectively, into the rats. Empty EVAc cylinders were fabricated by the same procedure. All the discs were exposed to UV irradiation for 1–2 h prior to implantation.

PCPP:SA in a 20:80 formulation was obtained from Nova Pharmaceutical Corporation, Baltimore, MD. Empty PCPP:SA discs (3 mm in diameter; 11.5 mg) and 30% loaded BCNU-PCPP:SA discs of the same size were synthesized by melt polycondensation, dissolved in methylene chloride, dried, and formulated into discs by compression molding as described by Leong *et al.* (10, 11). The polymers were kept at –35° until the day of implantation and sterilized by exposure to UV light for about 1 h.

s.c. Tumor Implantation and Treatment

The left flanks of 90 anesthetized rats were shaved and prepared in sterile fashion with 70% ethyl alcohol and povidone-iodine solution. The tumor was induced by the s.c. injection of 0.1 ml of 9L gliosarcoma homogenate by use of a 22-gauge needle. Tumor growth was documented by measuring the length and width of the tumors with dial calipers twice weekly (KWB Swiss, type 5921; Thomas Scientific, Swedesboro, NJ). Tumor volume was calculated by the formula for the approximation of the volume of a spheroid:

$$\text{Volume} = \frac{\text{Length} \times \text{Width}^2}{2}$$

(14, 15). Treatment was initiated when the tumors reached a calculated mean volume of about 600 mm³. There were no significant differences among the volumes of the four experimental groups at the time treatment was initiated. The animals were anesthetized, both flanks were shaved and prepared with 70% ethyl alcohol and povidone-iodine solution, and small incisions were made above the tumor on the left flank and on a similar location on the contralateral flank. For the controlled delivery of BCNU, 30% BCNU-EVAc discs weighing about 10 mg (containing a total dose of BCNU of 15 mg/kg) were implanted either next to the tumor or in the contralateral flank. Similar EVAc discs without BCNU were used as controls. The wounds were closed with surgical clips. An i.p. injection of 0.1 ml of ethyl alcohol:normal saline (1:1, v/v) containing 30 mg/ml of BCNU was administered within 5 min of preparation to one group and the same volume of the vehicle was administered to the other groups. When the tumor reached a volume of about 24 cm³,

the animals were anesthetized and then sacrificed by the i.p. injection of T-61 Euthanasia Solution (Taylor Pharmacal Company, Decatur, IL). The control group, the two systemic groups, and the local therapy group were sacrificed 18, 28, and 39 days after tumor inoculation, respectively.

i.c. Tumor Implantation and Treatment

The heads of 96 anesthetized rats were shaved and prepared in sterile fashion with 70% ethyl alcohol and povidone-iodine solution. For i.c. implantation, 9L gliosarcoma fragments measuring approximately 2 × 2 × 1 mm³ were excised at the time of implantation. Using microsurgical technique, a midline incision was made on the posterior aspect of the head and the periosteum was swept laterally to expose the sagittal, coronal, and lambdoidal sutures. A 3-mm burr hole was made over the left parietal region with its center 5–6 mm behind the coronal suture and 3–4 mm lateral to the sagittal suture. A cruciate incision was made on the dura. The underlying cortex was aspirated gently until the vascular junction between the posterior thalamus and the anterior superior colliculus was exposed. Bleeding from the resected cortical surface was controlled by cauterization with a silver nitrate applicator (Foster Medical Corporation, Dedham, MA). Once the bleeding stopped, the tumor fragment was transferred into the cortical defect over the brainstem. The wound was irrigated copiously and the skin edges were approximated with surgical clips. The rats in the control groups underwent the same i.c. procedure but did not receive tumor implants. The animals were allowed to wake up spontaneously and were returned to their cages when fully active.

On the fourth day after tumor implantation, the rats were randomized to one of eight experimental groups (Table 2) for initiation of treatment and reoperated for the insertion of the polymeric discs. The wound clips were removed and the skin was prepared in sterile fashion as described above. The wound was reopened and the burr hole was identified. Either 30% BCNU-EVAc discs weighing 11.5 mg or similar EVAc discs without BCNU were inserted through the craniotomy defect into the cerebral cortex. Intracranial implantation in rats of EVAc polymers 30% loaded with BCNU results in delivery of the total dose in about 9 days (13). An i.p. injection of 0.2 ml of ethyl alcohol:normal saline (1:1, v/v) containing 17.3 mg/ml of BCNU was administered within 5 min of preparation to the 9L/i.p. BCNU and i.p. BCNU groups (see Table 2) and the same volume of the vehicle was administered to the other six groups. The wound was reclosed with surgical clips and the animals were returned to their cages when fully awake.

The rats were examined twice daily with particular attention given to behavioral changes manifested by decreased alertness, passivity, impaired grooming, unkempt appearance, restlessness, irritability, or fearfulness and to neurological deficits as manifested by focal motor deficits or gait disturbances (16). Characteristically, 12–24 h prior to death from the i.c. tumor, the animals became lethargic and passive, developed a scruffy coat and lacrimal debris around the eyes, manifested an ataxic gait, or, in the advanced stages, displayed extensor posturing of the hind legs. The animals were sacrificed when at least four of these six signs appeared.

The rats were sacrificed by i.p. administration of anesthesia as described above followed by i.p. injection of T-61 Euthanasia Solution (Taylor Pharmacal Company). In all cases, a full autopsy was conducted to determine the cause of death. The brain, lungs, liver, stomach-duodenum, and kidneys were placed in buffered formalin for 10–14 days, embedded in paraffin, sectioned with a microtome, and stained with hematoxylin and eosin for histological examination.

Statistical Analysis

For the s.c. experiment, the efficacy of each therapeutic arm was assessed using a calculated value derived from the tumor growth delay assay (17, 18). The time required for each individual tumor to reach a volume of 6000 mm³ (*T*₆₀₀₀) was calculated as follows. The exponential growth curve for each tumor, described by the general exponential function

$$V = Ae^{Bt}$$

where *v* is tumor volume, *t* is time, and *A* and *B* are constants, was linearized

by taking its logarithm, yielding an equation in the form of a linear function described by the equation

$$\ln v = \ln A + Bt$$

Using linear regression analysis, A and B were determined and then used to calculate the time at which each tumor reached 6000 mm³ (T_{6000}) (19, 20). The T_{6000} values were then subjected to nonparametric single factor analysis of variance by the Kruskal-Wallis test and the Newman-Keuls nonparametric analogue for multiple comparisons (19). For the i.c. experiment, the survival values were analyzed by similar means (19).

RESULTS

The regional s.c. delivery of BCNU via EVAc (ipsilateral BCNU-EVAc group) resulted in a significant delay of 16.3 ± 1.3 (SEM) days ($P < 0.05$) in the growth of the 9L gliosarcoma compared to the growth delays with either the systemic i.p. administration (i.p. BCNU group) (11.2 ± 1.1 days) or the systemic s.c. controlled release administration (contralateral BCNU-EVAc group) (9.3 ± 1.3 days) of the same dose of BCNU (Fig. 1; Table 1). No significant difference was found between the T_{6000} values of the two systemic treatment groups.

The i.c. delivery of BCNU via either EVAc (9L/BCNU-EVAc group) or PCPP:SA polymers (9L/BCNU-PCPP:SA) resulted in a significant increase (5.4- and 7.3-fold; $P < 0.05$) in mean survival of rats bearing the 9L gliosarcoma compared to the systemic i.p. administration of the same BCNU dose (9L/i.p. BCNU group) (2.4-fold) (Fig. 2; Table 2). Moreover, five animals (42%) in the 9L/BCNU-EVAc group and two (17%) in the 9L/BCNU-PCPP:SA group survived to the end of the experiment (125 days); no viable tumor was found in any of these long-term survivors.

The survival figures in the three i.c. groups with the 36 animals that did not receive tumor implants assessed the toxicity of the two ther-

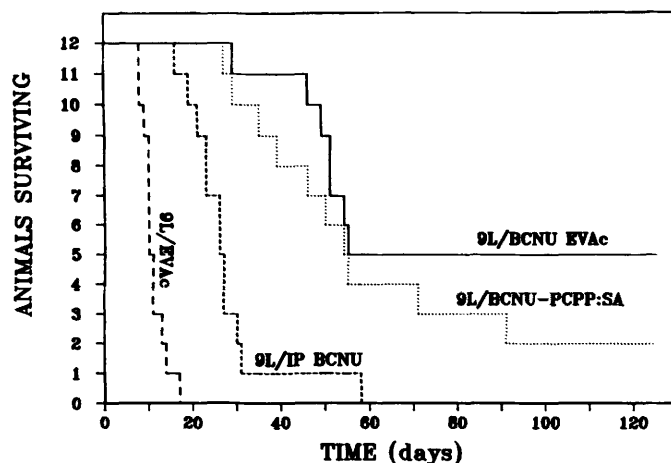


Fig. 2. Survival curves of the groups bearing i.c. tumors treated with either systemic (9L/i.p. BCNU) or interstitial i.c. (9L/BCNU-EVAc and 9L/BCNU-PCPP:SA) BCNU.

apeutic regimens (Fig. 3). All the rats in the control group without BCNU survived to the end of the experiment at 125 days. By contrast, 10 of 12 animals died prematurely after the systemic administration of BCNU (i.p. BCNU group) and 8 of 12 animals died prematurely after the interstitial i.c. delivery of BCNU (i.c. BCNU group). The mean survival of the i.c. BCNU group was higher than that of the i.p. BCNU group, but the difference was not significant. No obvious differences in behavioral changes or neurological deficits were noted between the two groups. Histopathological changes consisting of pulmonary changes (interstitial pneumonitis and fibrosis) and hepatic changes (small scattered microabscesses) were observed in all groups treated with BCNU but were not detected in untreated animals. In general, these findings, which were identified in both long- and short-term survivors, were relatively mild and could not be correlated with premature deaths in any of the groups.

DISCUSSION

This study illustrates certain advantages of the interstitial s.c. and intracerebral controlled release of BCNU for treatment of the 9L gliosarcoma. The administration of the same BCNU dose is more effective when delivered directly into the site of the growing tumor than when administered systemically, as evidenced by the increased survival of animals treated i.c. in this manner and by the delayed growth of tumor in animals with s.c. implants. Despite the high doses of BCNU released i.c., the toxicity of interstitial therapy with BCNU is comparable to that of the systemic administration of this agent. The results of the experiments described here allowed us to proceed with a phase I-II clinical trial in which patients with recurrent malignant astrocytomas underwent surgery for placement of polymers containing BCNU (21). A multiinstitutional phase III placebo-controlled clinical trial is currently in progress to evaluate this therapeutic approach in patients with recurrent gliomas.

The polymers used in this study are representative of two major categories of polymeric formulations currently available for controlled

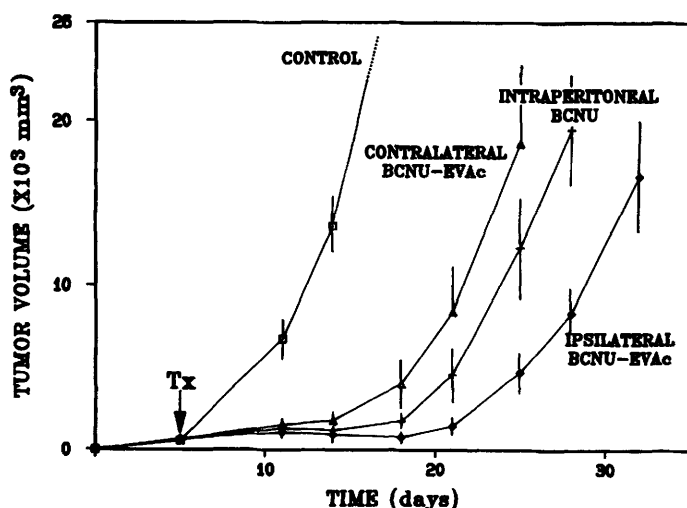


Fig. 1. Tumor growth curves of the four s.c. experimental groups. Bars, SEM. Tx, time at which treatment was initiated with BCNU.

Table 1 Study design: s.c. experimental groups

The control group had 21 rats and the other 3 groups had 23 rats each. T_{6000} values are presented as mean \pm SEM.

Group label	Tumor flank implant	Contralateral flank implant	i.p. injection	T_{6000}^a (days)
Ipsilateral BCNU-EVAc	BCNU in EVAc	Empty EVAc	Vehicle	28.6 ± 1.0
Contralateral BCNU-EVAc	Empty EVAc	BCNU in EVAc	Vehicle	21.6 ± 1.0
i.p. BCNU	Empty EVAc	Empty EVAc	BCNU	23.5 ± 0.8
Control	Empty EVAc	Empty EVAc	Vehicle	12.3 ± 0.3

^a T_{6000} , time in days for tumors to reach a volume of 6000 mm³; BCNU in EVAc, s.c. implantation of a 10-mg EVAc disc containing 3.0 mg of BCNU (30% loading of BCNU); empty EVAc, s.c. implantation of a 10-mg EVAc disc; BCNU, i.p. injection of 0.1 ml of ethyl alcohol:normal saline (1:1, v/v) containing 30 mg/ml of BCNU; vehicle, i.p. injection of 0.1 ml of ethyl alcohol:normal saline (1:1, v/v) without BCNU.

Table 2 Study design: intracranial experimental groups

Each group had 12 rats. Survival values are presented as mean \pm SEM. Two rats that died perioperatively were excluded from the study (one from an i.c. hemorrhage and the other from an i.c. abscess).

Group label	9L ^a glioma	Intracranial polymer implant	i.p. injection	Survival (days)
9L/EVAc	Yes	Empty EVAc	Vehicle	10.9 \pm 0.8
9L/PCPP:SA	Yes	Empty PCPP:SA	Vehicle	11.6 \pm 0.7
9L/i.p. BCNU	Yes	Empty EVAc	BCNU	27.3 \pm 3.1
9L/BCNU-EVAc	Yes	BCNU in EVAc	Vehicle	80.0 \pm 11.6
9L/BCNU-PCPP:SA	Yes	BCNU in PCPP:SA	Vehicle	62.3 \pm 9.9
Control	No	Empty EVAc	Vehicle	(125)
i.p. BCNU	No	Empty EVAc	BCNU	63.3 \pm 8.0
i.c. BCNU	No	BCNU in EVAc	Vehicle	92.7 \pm 8.9

^a 9L, i.c. implantation of the 9L gliosarcoma at first operation; empty EVAc, i.c. implantation at second operation of an 11.5-mg EVAc disc; vehicle, i.p. injection at second operation of 0.1 ml of ethyl alcohol and 0.1 ml of normal saline; empty PCPP:SA, i.c. implantation at second operation of an 11.5-mg PCPP:SA disc; BCNU, i.p. injection at second operation of 3.5 mg of BCNU in 0.1 ml of ethyl alcohol and 0.1 ml of normal saline; BCNU in EVAc, i.c. implantation at second operation of an 11.5-mg EVAc disc containing 3.5 mg of BCNU (30% loading of BCNU); BCNU in PCPP:SA, i.c. implantation at second operation of an 11.5-mg PCPP:SA disc containing 3.5 mg of BCNU.

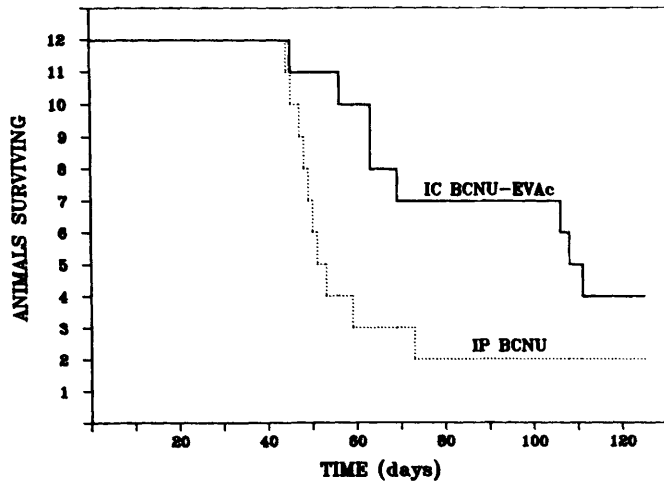


Fig. 3. Survival curves of the groups exposed to BCNU systemically (i.p. BCNU) or interstitially (i.c. BCNU-EVAc).

release (9). EVAc is a biologically inert polymer capable of releasing a wide spectrum of agents in a diffusion-regulated, controlled fashion (7, 8). The release kinetics of this polymer can be tailored by modifying the nature of the drug or the structure of the polymer (22–24). PCPP:SA, a biodegradable polyanhydride (25), releases its drug as its polymeric matrix is hydrolyzed. Like EVAc, PCPP:SA has highly reproducible release kinetics, and its degradation products are bio-compatible with the brain of rodents and humans. The release kinetics of PCPP:SA also can be modified by changing the ratio of the two monomeric components PCPP and SA. Thus the drugs can be released for periods ranging from days to years. These synthetic polymers provide the added advantage of being fully biodegradable, thus obviating the problems associated with permanent implants (10–12, 21).

Interstitial drug delivery with polymeric carriers may enhance the pharmacological alternatives for the treatment of malignant tumors. This regional approach may prove particularly useful in the treatment of malignant astrocytomas, many of which present as local disease and most of which recur locally, within a 2-cm margin of the primary site (26–28). Chemotherapeutic agents that, unlike BCNU, are excluded by the blood-brain barrier and thus have not proven useful in the treatment of gliomas, can now be incorporated in the polymers and implanted at the site of tumor growth. Since the bioavailability to the central nervous system of systemically administered drugs is limited by the presence of the blood-brain barrier (29), the current list of useful chemotherapeutic agents for the treatment of brain tumors is limited to lipid-soluble and relatively non-ionized drugs (30). Even when appropriate agents are used, high doses are required systemically to reach therapeutic levels in the brain, resulting in systemic toxicity but unfortunately without significant improvement in survival

(31). The use of polymer delivery systems may obviate these problems and therefore broaden the therapeutic armamentarium.

Polymeric vehicles may have numerous applications in neuroscience, since systemic pharmacological access to the nervous system remains a major obstacle in the treatment of most neuropathological conditions. Not only can a wider variety of chemotherapeutic agents be used to treat brain tumors when these agents are delivered by polymers, but biological response modifiers, such as angiogenesis inhibitors (32, 33), can also be administered in this fashion. In general, polymeric carriers may prove ideally suited for therapy within the nervous system; interstitial delivery of antibiotics for cerebral infections (33), of corticosteroids for control of cerebral edema (34), of vasodilators for vasospasm (35), and of adrenergic/cholinergic agents for movement disorders (36, 37) are only a few of the emerging applications of polymeric carriers in the brain. The concept of interstitial controlled delivery with polymers, however, may prove useful outside the central nervous system, since regional therapy may be of value in other organs when localized and controlled release of drugs over prolonged periods is desirable.

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