

The intracerebral distribution of BCNU delivered by surgically implanted biodegradable polymers

STUART A. GROSSMAN, M.D., CARLA REINHARD, M.S., O. MICHAEL COLVIN, M.D., MARK CHASIN, PH.D., ROBERT BRUNDRETT, PH.D., RAFAEL J. TAMARGO, M.D., AND HENRY BREM, M.D.

Departments of Oncology and Neurosurgery, The Johns Hopkins Medical Institutions, and Nova Pharmaceutical Corporation, Baltimore, Maryland

✓ The local concentration and distribution of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) within normal brain tissue were studied following surgical implantation of biodegradable polymer containing BCNU in New Zealand White rabbits. Cylindrical discs of poly(bis(p-carboxyphenoxy)-propane:sebacic acid) copolymer in a 20:80 formulation were made containing [³H]-inulin or [³H]-BCNU labeled in the methylene hydrogens of the chloroethyl groups. These were implanted in the brains of 56 New Zealand White rabbits. The animals were sacrificed 3, 7, 14, or 21 days later and the brains were rapidly removed, frozen, and prepared for quantitative autoradiography. Autoradiographs from coronal sections bisecting the polymer were analyzed to determine both the proportion of the brain section exposed to the tracer and the local drug concentrations as a function of distance from the polymer. Tritiated BCNU was also injected directly into the brains of eight additional rabbits, and local brain concentrations were studied over time.

The results of this study demonstrate that approximately 50% of the area of the brain sections was exposed to radiolabeled compound 3 days after BCNU-polymer implantation, 15% at 7 days, and less than 10% at 14 and 21 days. Polymer discs containing 600 µg BCNU generated 6 mM concentrations of BCNU in brain tissue 10 mm from the polymer at 3 and 7 days. Pharmacological studies demonstrated that approximately 25% of the tritium label was associated with intact BCNU 3 days following polymer implantation. Radiolabeled inulin delivered by polymer remained dispersed throughout the ipsilateral hemisphere for 14 days. Direct injection of [³H]-BCNU into brain parenchyma resulted in widely distributed tracer at 1 and 3 hours with rapid disappearance thereafter. It is concluded that local delivery of BCNU to brain tissue with this polymeric drug delivery system results in sustained high local concentrations of BCNU which may be of value in the treatment of patients with brain tumors.

KEY WORDS • brain neoplasm • BCNU • chemotherapy • drug delivery • autoradiography • rabbit

MALIGNANT brain tumors in adults are resistant to surgery, radiation therapy, and chemotherapy.^{25,28} Despite aggressive multimodality treatment, these tumors tend to recur within centimeters of their original location.²² In an effort to decrease local recurrences, recent efforts have focused on increasing the delivery of radiation and chemotherapy to tumor-bearing regions.^{3,19,26} Implanted biodegradable polyanhydrides that release chemotherapeutic agents in an active form for prolonged periods of time could improve local control in patients with these tumors. The biocompatibility of these polymers in brain tissue has been demonstrated in animal models, as has their ability to release biologically active nitrosoureas for extended periods of time.^{5,7,8,10,27,29,43,44,49} Several therapeu-

tic advantages could result from placement of drug-imregnated polyanhydrides into the surgical cavity after debulking an intracerebral tumor. High local concentrations of antineoplastic agents would be delivered to tumor and adjacent tissue, systemic toxicity of these chemotherapeutic agents would be minimized, and the inconsistent delivery of systemically administered agents through a variably intact blood-brain barrier would be eliminated.

The purpose of the present studies was to define the regional distribution and local concentrations of 1,3-bis(2-chloroethyl)-nitrosourea (BCNU) following implantation of BCNU-containing polyanhydride polymer in the brain of normal rabbits. The results obtained with this small, lipid-soluble compound delivered by

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the polymer were also to be compared to the distribution of inulin (a large, inert, water-soluble compound) released from the polymer, and of BCNU following direct intracranial injection.

Materials and Methods

Synthesis of [³H]-BCNU

Radiolabeled BCNU was synthesized for the quantitative autoradiographic studies. Five mCi (12 Ci/mmol) of 1-[³H]-2-aminoethanol hydrochloride was added to 20 mg (0.3 mmol) of 2-aminoethanol in 5 ml of water. This mixture was cooled to 0°C, and 40 mg (0.38 mmol) of 2-chloroethyl isocyanate was added; the mixture was then stirred for 1 hour and evaporated to dryness under reduced pressure. Next, 6 ml of chloroform and 0.5 ml of thionyl chloride were added and the mixture was refluxed for 1 hour. The solvent was removed under reduced pressure and 3 ml of formic acid (88%) was added. This was cooled to 0°C, and 350 mg (5 mmol) of sodium nitrite in 1 ml of water was added with stirring over 30 minutes. A blue color persisted for several minutes after the last addition. After another hour of stirring, an additional 10 ml of water was added. Three extractions were performed with methylene chloride, and the combined extracts were dried with sodium sulfate and evaporated under reduced pressure. The resulting product was chromatographed on a 1.4 × 14-cm silica gel column with chloroform. The yellow band was collected, evaporated to an oil under reduced pressure, seeded with a minute crystal of unlabeled BCNU, and allowed to crystallize overnight at 10°C. A sample chromatographed on silica gel with benzene gave the same R_f (0.68) as authentic BCNU. The yield of [³H]-BCNU was 70 mg (0.35 mmol) with a total activity of 2.6 mCi, for a specific activity of 7.5 mCi/mmol. This [³H]-BCNU was incorporated into the polymer. A second batch of [³H]-BCNU yielding a specific activity of 0.1 mCi/mmol was synthesized using the same procedures for the direct stereotactic injection of [³H]-BCNU into the rabbit brains.

Polymer Preparation

The carboxyphenoxypropane monomer and prepolymer, the sebacic acid (SA) prepolymer, and the poly(bis(p-carboxyphenoxy)-propane (PCPP):SA) polymer were prepared as previously described.^{7,11} A PCPP:SA ratio of 20:80 was used to synthesize the polymers. The tritiated compounds were loaded into the polymers by either trituration or solution; both methods are described below. Polymers loaded with 2.5%, 5%, or 10% BCNU were made by both of these methods. These polymer discs contained 300, 600, or 1200 µg of BCNU, and showed 10.56, 21.12, or 42.24 µCi, respectively. The inulin-containing polymer discs were made only by the trituration method and contained only one concentration of [³H]-inulin (0.2 mg, 40 µCi). The specific activity of the [³H]-BCNU incorporated into the polymer was 7.5 mCi/mmol and that

of [³H]-inulin was 889 mCi/mmol. Radiochemical purity tests of the [³H]-inulin demonstrated that the materials administered were more than 95% pure.

Trituration Method. The BCNU-containing discs with a loading factor of 2.5% were prepared by trituring 5.1 mg [³H]-BCNU with 198.9 mg of PCPP:SA in a 20:80 ratio. For a loading factor of 5%, 10.2 mg [³H]-BCNU was triturated with 193.8 mg PCPP:SA, and for 10% loaded discs, 20.4 mg [³H]-BCNU was triturated with 183.6 mg of the polymer. Individual 12-mg portions of this [³H]-BCNU:polymer mixture were weighed and placed into a die with an internal diameter of 3 mm. Two such dies were then placed into a Carver press and compressed at 138,000 psi for 4 minutes. At the conclusion of compression, the cylindrical discs weighed 12 mg and measured 3 mm in diameter and 1 mm thick. Each disc was immediately placed into an aluminum foil pouch* purged with dry argon, and heat-sealed. The pouch was then placed into a larger outer pouch of the same material which was also purged with dry argon and heat-sealed. These were sterilized with 2.5 Mrad of gamma radiation and stored at -25°C until used. Each inulin-containing disc contained 40 µCi of [³H]-inulin. To prepare these discs, 3.8 mg [³H]-inulin was triturated with 200.3 mg PCPP:SA. Aliquots of 12 mg each were individually weighed, pressed, and packaged as above. This method resulted in a suspension of BCNU or inulin particles in the polymer matrix.

Solution Method. Polymeric discs containing [³H]-BCNU produced by the solution method were prepared using solutions of [³H]-BCNU (120 mg) dissolved in methylene chloride (10 mg/ml), PCPP:SA in a ratio of 20:80 (500 mg) dissolved in 10 ml methylene chloride, and unlabeled BCNU (10 mg) dissolved in 1.0 ml methylene chloride. These were kept on ice during disc preparation. The 2.5% loaded discs were made with 2.8 ml of the polymer solution mixed with 360 µl of the [³H]-BCNU solution. The 5% loaded discs required 2.7 ml of the polymer solution and 720 µl of the [³H]-BCNU solution, and the 10% loaded discs used 2.6 ml of the polymer solution mixed with 1.4 ml of the [³H]-BCNU solution and 310 µl of the unlabeled BCNU solution. In each case, the solvent was removed by vacuum and the remaining material in each beaker was scraped and ground into small particles. Aliquots of 12 mg each were individually weighed, pressed, and packaged as described above for the trituration method. This procedure resulted in a solid solution of BCNU dissolved in the polymer. The polymers were sterilized by exposure to ultraviolet light for 1 hour.

Administration of Radiolabeled Compounds

All animal experiments were performed with the approval of the Johns Hopkins Committee on Animal Experimentation. Aseptic surgical techniques were followed for all procedures.

* Aluminum foil pouches manufactured by Ivers-Lee, West Caldwell, New Jersey.

Direct Stereotactic Injection of [³H]-BCNU. Male New Zealand White rabbits, each weighing 3 to 4 kg, were anesthetized with 250 mg xylazine and 250 mg ketamine administered intramuscularly. A 3-cm frontoparietal sagittal incision was made and the intersection of the coronal and sagittal sutures was exposed (the horizontal zero-point for stereotactic injection). A 4-mm burr hole was drilled 5 mm lateral from the bregma and 1 mm anterior to the coronal suture. Following placement of the rabbit's head in a stereotactic frame, a No. 27 cannula was introduced into the brain parenchyma over a period of 2 minutes to a depth of 3 mm. The delivery of [³H]-BCNU was initiated 3 minutes after the cannula was fully inserted. Each rabbit received a total volume of 5 μ l (40 μ Ci) which was drawn from a solution comprising 17 mg [³H]-BCNU (9.1 mCi/mmol) in 70 μ l ethanol and 20 μ l of a 2% Evans blue solution. This was administered at a rate of 0.5 μ l/min which was controlled with a syringe support, caliper advance, and a stopwatch. The cannula was slowly withdrawn 4 minutes after the drug was delivered and the skin was closed with surgical staples. The animals were allowed to awaken spontaneously and were carefully observed in their cages. The rabbits were sacrificed 1, 3, 24, or 72 hours after injection, with an intramuscular injection of xylazine and ketamine followed by 21 ml of intravenous euthanasia solution.[†] The brain was quickly removed and frozen in hexane at -35°C, then set in a thin coat of embedding matrix[‡] and stored in a -70°C freezer for further processing.

Surgical Implantation of the Polymer. Surgical implantation of polymer containing radiolabeled BCNU or inulin was performed using the same anesthesia and size and location of burr holes as described above. A slit 3 to 5 mm deep was made in the dura and brain parenchyma with a No. 11 scalpel blade. The cylindrical polymeric disc was inserted into the brain parenchyma with a pair of jewelers forceps until it was no longer visible. Once the bleeding spontaneously ceased, the skin was closed with surgical clips. Rabbits receiving radiolabeled BCNU- and inulin-containing polymer were sacrificed 3, 7, 14, or 21 days following surgical implantation. The methods used for euthanasia, tissue harvesting, and freezing were as described above.

Preparation of Tissues for Quantitative Autoradiography

The frozen and embedded brain was mounted on a cryostat chuck with embedding matrix and cut in 10- μ sections in a Harris cryostat at -20°C. Two tissue sections were saved at 200- μ intervals throughout the brain. One was prepared for histological studies, while the other was used for quantitative autoradiography. The histological section was placed on a gelatin-coated

microscope slide and stained with hematoxylin and eosin. The autoradiographic section was harvested on a glass slide warmed to 60°C. Commercially prepared tritium standards 10 μ thick, comprising eight levels of activity ranging from 6660 to 244,200 disintegrations per minute (dpm)/mg[§] and the quantitative autoradiographic sections were placed against tritium-sensitive sheet film in an x-ray cassette and exposed for 2 weeks at room temperature. The film was developed for 5 minutes at 22°C, immersed for 30 seconds in a stop bath, 5 minutes in fixer, and 20 minutes in filtered flowing water at 22°C, briefly rinsed, and allowed to dry. A representative histological section and the corresponding digitized autoradiograph are shown in Fig. 1.

Quantitative Autoradiography Data Analysis

The autoradiographs and tritium standards were digitized with an automated biological/medical image analysis system.^{||} Optical densities (OD's) from the autoradiography film exposed to the tritium standards were used to generate curves to convert OD data into dpm/mg tissue. A power function equation in OD provided the highest coefficient of determination ($r^2 = 0.994$). Using the specific activity of the [³H]-BCNU, dpm/mg tissue were converted into pmol BCNU/mg tissue and subsequently into molar concentrations of BCNU. A coronal brain section that bisected the polymer or direct injection site in each animal was chosen for detailed quantitative analysis. The proportion of this tissue section exposed to radiolabeled tracer and the concentrations of [³H]-BCNU adjacent to the polymer or injection site were evaluated in each of the 64 animals studied.

Percentage of Tissue Section Exposed to Tracer
The total area of the section of brain that bisected the polymer was determined from the slides prepared for histological study. This tissue section was digitized, the outermost border of the brain was outlined with a cursor, and the overall area of each brain section was determined using the image analysis system. The autoradiographic image was then analyzed to determine the percentage of each brain section exposed to the radiolabeled compound. This was defined as the area of the brain which had an OD on the autoradiography film of more than 2 standard deviation (SD) above the background OD of the film. The mean OD and SD of the film immediately surrounding the brain images were calculated. Regions of the brain section with an OD greater than or equal to the mean plus 2 SD of the background film OD were identified using the image analysis system, and the percentage of the total area of the brain section which this represented was calculated. A level of 2 SD above background OD was chosen to ensure that measurements made for BCNU and inulin

[†] Euthanasia solution (T-61) manufactured by Taylor Pharmaceutical Co., Decatur, Illinois.

[‡] Embedding matrix (M-1) manufactured by Lipshaw Manufacturing Co., Detroit, Michigan.

[§] Tritium standards manufactured by Amersham Corp., Arlington Heights, Illinois.

^{||} Image analysis system manufactured by Loats Associates, Inc., Westminster, Maryland.

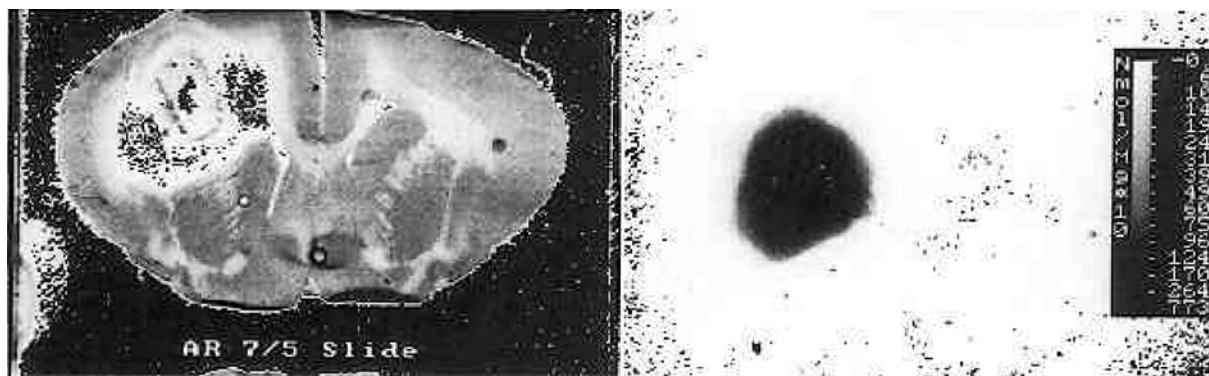


FIG. 1. A representative histological section (*left*) and the corresponding digitized autoradiograph (*right*) from a rabbit brain 7 days after implantation of polymer containing 1200 μg of radiolabeled BCNU. These are shown at the same magnification.

had less than a 5% chance of arising due to statistical variation of the film. In this way, the background OD of the autoradiography film and increased OD secondary to radiolabeled tracer in the brain were carefully separated.

Local Concentrations of $[^3\text{H}]\text{-BCNU}$. The average concentration of $[^3\text{H}]\text{-BCNU}$ in specific regions of the brain was determined by outlining these regions of interest on the digitized autoradiographic images. Optical density data from these regions were converted to dpm/mg tissue and to regional concentrations of BCNU as described above. Anatomical correlations were made using the corresponding brain sections stained with hematoxylin and eosin and an atlas of the anatomy of the rabbit brain.¹⁵ Drug concentrations were then analyzed as a function of distance from the polymer. A line was defined on the digitized autoradiographic image which passed from the lateral borders of the brain section through the center of the polymer. Drug concentrations were assessed along this line and analyzed as a function of distance from the surface of the polymer.

Pharmacological Identification of Radioactive Autoradiograph Material

Pharmacological studies were performed on a brain that had a polymer containing 1200 μg $[^3\text{H}]\text{-BCNU}$ implanted 3 days prior to sacrifice. The brain was harvested and processed using the methods described above. Punch biopsies were taken from a coronal slice of the brain 0.3 cm thick which encompassed the center of the polymer. Biopsies were obtained at distances of 1 to 3 mm from the edge of the polymer at approximate intervals of 120° around the periphery. Each sample was minced and extracted with ethanol, and the residual tissue was separated by centrifugation. Aliquots of the ethanol extract and the residual tissue were analyzed for tritium by liquid scintillation counting. The ethanol extract was evaporated to dryness, dissolved in a small volume of ethanol containing nonradioactive BCNU,

and spotted on a plastic thin-layer chromatography sheet. The chromatogram was developed with benzene and the BCNU spot was localized with fluorescence. Each thin-layer chromatography lane was cut into sections and the tritium radioactivity on each section was determined by liquid scintillation counting. Compounds remaining at the origin were considered polar decomposition and/or metabolic products of the BCNU.

Results

Quantitative autoradiography was performed on a total of 64 New Zealand White rabbits which received polymer implants or direct injections of radiolabeled BCNU. Two rabbits were studied at each time point (3, 7, 14, and 21 days after polymer implantation) for the 300-, 600-, and 1200- μg BCNU-loaded discs prepared by the trituration method, for the 300-, 600-, and 1200- μg BCNU-loaded polymers made by the solution method, and for the inulin-loaded discs. Eight additional rabbits were studied following direct injection of $[^3\text{H}]\text{-BCNU}$ into the brain.

Area of Brain Exposed

The percentage of the brain sections exposed to the radiolabeled compound in the animals that received the 1200- μg trituration and solution polymers, the triturated inulin polymer, and the direct injection of $[^3\text{H}]\text{-BCNU}$ as a function of time are shown in Fig. 2. Each of these animals received 40 μCi of tritium. Following the direct injection of $[^3\text{H}]\text{-BCNU}$, labeled tracer was rapidly seen throughout the ipsilateral hemisphere but quickly cleared from the brain. At 24 hours after injection, only 15% of the area of the brain section was exposed to drug and, by 72 hours, this had fallen further. In contrast, 72 hours after implantation of polymer prepared by either method, 40% of the area of the brain section was exposed to the tracer and the fall in exposure to the 15% level did not occur for 180 to 350 hours after implantation. The results observed with the BCNU polymer discs made by either the trituration or solution

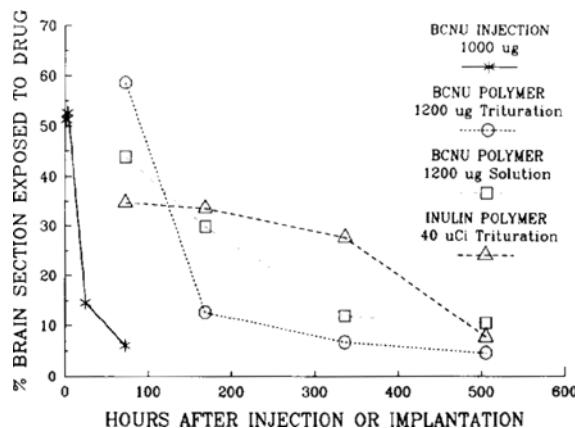


FIG. 2. Graph showing distribution of $[^3\text{H}]$ -BCNU and inulin delivered by polymer and $[^3\text{H}]$ -BCNU by direct injection into rabbit brain. The percentage of the area of brain sections exposed to the radiolabeled compound in animals is given following administration of 1200 μg trituration (circles) and solution (squares) polymers, triturated inulin polymer (triangles), and direct injection of $[^3\text{H}]$ -BCNU (stars). These data are displayed as a function of time following implantation or injection for two rabbits per time point. Each animal received 40 μCi tritium.

methods were not significantly different. The exposure of the brain to $[^3\text{H}]$ -inulin released from the polymer appeared relatively constant for approximately 2 weeks (Fig. 2). During this time more than 30% of the area of the brain section bisecting the polymer contained significant radiolabeled inulin; thereafter, the total area of the brain section exposed to inulin began to diminish.

The total area of the brain sections exposed to $[^3\text{H}]$ -BCNU released from polymer and the average concentrations of BCNU contained within this area as a function of BCNU loaded into the polymer and time are shown in Table 1. Although the average concentration appears to increase with time following polymer implantation, this is due to a progressively diminishing region of distribution within the brain section. At the later times, the area of higher activity is close to or within the polymer.

Concentration Profiles

A profile of concentrations as a function of distance from the implanted polymer was studied in each animal. This profile consisted of several components: the film background, a region containing tracer adjacent to the polymer, a large amount of tracer residing within the polymer, and a central region where a portion of polymer is often physically removed from the tissue section at the time of the cryostat sectioning. The region with tracer adjacent to the polymer was studied to determine local BCNU concentrations. Three days after disc implantation, this area of activity extended approximately 12 mm from the polymer loaded with 300 μg BCNU. The average BCNU concentration within this region was approximately 3 mM. Seven days following implantation, this area was more difficult to de-

TABLE I
Distribution and concentration of $[^3\text{H}]$ -BCNU administered by polymer in normal rabbit brain*

Time Postimplant	% Brain Section Exposed	Average Concentration (mM BCNU)
300- μg BCNU polymer		
day 3	34.0	1.9
day 7	5.2	5.8
day 14	4.1	5.4
day 21	2.4	7.4
600- μg BCNU polymer		
day 3	30.0	2.5
day 7	20.2	5.3
day 14	4.8	7.7
day 21	4.2	7.1
1200- μg BCNU polymer		
day 3	58.5	2.9
day 7	18.0	6.3
day 14	7.0	7.6
day 21	4.5	7.3

* BCNU-loaded polymer prepared by trituration method. Values are average data for two animals sacrificed at each time point.

fine and extended less than 4 mm from the polymer. These findings persisted with minor changes to 21 days. The 600- μg BCNU discs generated a region of activity with a 6-mM concentration that extended approximately 10 mm from the polymer at Days 3 and 7 following implantation. On Day 14, this region was less evident and extended to a diameter of less than 3 mm. The 1200- μg discs generated a 12-mm zone of activity with concentrations in the 8-mM range. The diameter of activity was 5 mm at both 7 and 14 days following polymer implantation, and at 21 days it measured 3 mm. The diameter of the region with tracer, the BCNU concentration at the edge of this area, and the average concentration within this region 72 hours following polymer implantation in the animals receiving $[^3\text{H}]$ -BCNU polymer prepared by the trituration method are shown in Table 2.

Pharmacological Studies

Pharmacological studies performed 72 hours after polymer implantation demonstrated that all tritium activity present on the thin-layer chromatography was coincident with the BCNU standard or remained at the origin. Compounds at the origin were considered polar decomposition and/or metabolic products of BCNU. In this way, it was estimated that, at 72 hours following implantation of the $[^3\text{H}]$ -BCNU polymer into the brain, approximately 26% of the radioactivity was associated with intact BCNU, 24% with polar metabolites of BCNU, while 46% was bound to the tissue. The individual biopsy results and the average findings are shown in Fig. 3.

Histological Examinations

Frozen sections corresponding to those used for quantitative autoradiography were stained with hema-

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TABLE 2
Results 72 hours after implantation of BCNU-loaded polymer in rabbit brain*

BCNU Dose	Diameter of Tracer Surrounding Polymer (mm)	Concentration at Edge of Tracer Region (mM BCNU)	Average Concentration within Tracer Region (mM BCNU)
300- μ g BCNU polymer	12	0.55	3
600- μ g BCNU polymer	10	0.62	6
1200- μ g BCNU polymer	12	0.70	8

* BCNU-loaded polymer prepared by trituration method. Values are averages of two animals each.

toxylin and eosin and examined under a microscope. Brain sections from the animals with inulin-containing polymers appeared relatively normal. Direct injections of [³H]-BCNU resulted in some local necrosis and edema at the site of injection, which was most prominent at early time points. Histological abnormalities noted in the animals with BCNU-containing polymer discs were most prominent adjacent to the 1200- μ g loaded discs and least prominent adjacent to those loaded with 300 μ g of the drug. These brains had signs of local edema and necrosis which were most evident early and were resolving at the later times. These abnormal areas on the histological sections closely paralleled the regions of the brains that contained the highest concentrations of [³H]-BCNU as seen by quantitative autoradiography (Fig. 1). No difference was noted in the brains of animals receiving BCNU polymers prepared by the trituration or solution methods.

Discussion

Primary brain tumors are diagnosed in 8000 to 12,000 patients in the United States each year.³¹ Although surgery is an important treatment modality, tumor cells almost invariably remain after aggressive attempts at surgical removal of high-grade tumors in adults.²⁴ Radiation therapy prolongs survival, but tumor recurrence is usually evident in months.⁴⁷ The administration of systemic chemotherapy following surgery and radiation therapy adds little to survival in this patient population.^{25,40,45,46} The blood-brain barrier is frequently considered a major factor limiting the efficacy of systemically administered chemotherapeutic agents.^{2,36,38} Laboratory and clinical investigators have attempted to increase the delivery of antineoplastic agents to brain tumors using a variety of experimental approaches. Osmotic blood-brain barrier disruption, high-dose chemotherapy followed by autologous bone marrow rescue, and intra-arterial chemotherapy have demonstrated that brain tumors are potentially responsive to the delivery of high doses of chemotherapeutic agents.^{9,18,21,23,30,32,39,42} Unfortunately, considerable toxicity has been associated with these experimental therapies.

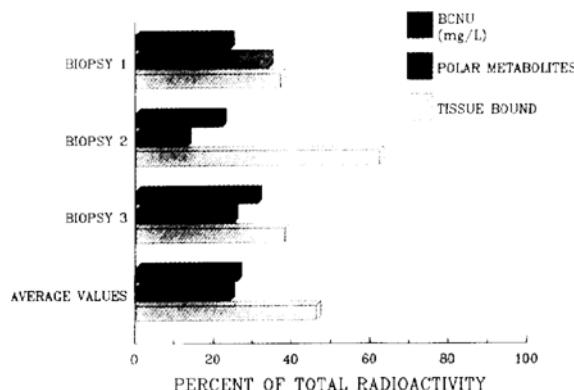


FIG. 3. Graph showing the percentage of radioactivity associated with intact BCNU, with polar metabolites of BCNU, or bound to the tissue 3 days after 1200 μ g BCNU polymer implantation in rabbit brain, as assessed by punch biopsies and thin-layer chromatography.

Other investigative studies are based on the observation that astrocytomas usually recur within centimeters of their original location despite aggressive surgery and radiation therapy.²² Interstitial radiation implants are being studied to determine if they reduce local recurrences.¹⁹ Chemotherapy has also been administered directly into brain tumors.^{4,12,14,37,48} The efficacy and toxicities of this approach have yet to be determined.

Polymeric Drug Delivery

This study examines the distribution of [³H]-BCNU delivered from polyanhydride polymer discs placed in the brains of normal rabbits. It compares this to the distribution of radiolabeled inulin delivered in a similar fashion and to direct intraparenchymal injections of the same dose of radiolabeled BCNU. The results indicate that BCNU is present within a local region of the brain for considerably longer after implantation of a BCNU-containing polymer than following the direct injection of this agent. Three days after implantation of the polymer, radiolabeled compound is extensively distributed within the ipsilateral hemisphere. However, the tracer is restricted to the area directly adjacent to the polymer 7 to 21 days after polymer insertion. Previous studies have shown that serum concentrations were extremely low and only detectable for the first 12 hours following insertion of polymer into brain.⁴⁹ Pharmacological correlations demonstrate that a sizable fraction of the tracer identified by quantitative autoradiography is associated with intact BCNU. The two different methods of polymer preparation studied produce similar results and both yield local concentrations of BCNU in excess of those required to kill glioblastoma cells in culture.¹ The direct injection of BCNU and the implantation of BCNU-containing polymer appear to cause a temporary, dose-related, local injury to the brain that was not observed in the animals receiving the inulin-loaded polymers. These observations,

combined with the finding that regions of edema and local necrosis correspond to areas containing the highest concentrations of the radiolabeled compound, suggest that the local brain injury is secondary to BCNU.

These results indicate that polymeric drug delivery may represent another approach to the treatment of primary brain tumors. The implantation of polymers containing BCNU at the site of a resected brain tumor could provide sustained high local BCNU concentrations which should not be associated with significant systemic toxicity. Polymers for sustained local drug release have been evaluated for the treatment of glaucoma, for contraception, and for the prevention of dental caries.^{13,16,17,34,41} Most clinically available polymeric drug delivery systems consist of hydrophilic matrices that absorb water and undergo homogeneous degradation.²⁰ These processes would result in inactivation of labile chemotherapeutic agents. Ideally, a polymeric drug delivery system for antineoplastic agents would be hydrophobic and would degrade primarily from the surface. This would maintain these drugs in a relatively "water-free" environment until the eroding border of the dissolving polymer reached them. The polyanhydride polymers used in our experiments meet these criteria. These biocompatible polymers release biologically active nitrosoureas for extended periods of time.^{5,7,10,27,44,49} The results of a Phase I-II study using BCNU-impregnated polymer implants in patients with recurrent astrocytomas have been reported and further clinical studies are in progress.⁶

Future Considerations

The treatment of intracranial neoplasms with chemotherapy administered by local implantation of polymers poses new dilemmas which must be considered. For example, the differences in the distribution of [³H]-BCNU and [³H]-inulin observed in this study could result from dissimilar release characteristics from the polymer or they could reflect differences in the clearance of these compounds from the brain once they are released from the polymer. Small, lipid-soluble agents that can pass through the blood-brain barrier, such as BCNU, may diffuse limited distances and be cleared from the brain rapidly. On the other hand, large, water-soluble compounds that cannot traverse an intact blood-brain barrier, such as inulin, may remain longer within the brain, diffuse more extensively, and be cleared to some extent by the cerebrospinal fluid. The movement of drugs through neoplastic tissues and regions where the blood-brain barrier is disrupted could also be significantly different than that through the normal brain tissue studied in our experiments. Thus, the integrity of the blood-brain barrier, the nature of the tissue to be treated, the molecular weight and lipid solubility of the chosen agent, and the physical properties of the polymer will all need to be considered in planning therapy for patients with brain tumors using this drug delivery system. In addition, the neurotoxicity of agents to be administered by intracranial polymer

must be carefully considered, as sustained high concentrations of these agents in the brain will result from this novel therapeutic approach.

Conclusions

Polyanhydride polymers represent an extremely versatile drug delivery system.^{4,29} They can be formulated to release drugs for periods ranging from days to years and to deliver radiosensitizers, glucocorticoids, or chemotherapeutic agents. Local delivery of these compounds could ultimately result in improved therapy for patients with primary brain tumors. Improved local control in patients with intracranial metastases is emerging as an important clinical challenge that may also be well suited to topical chemotherapy.^{33,35} A similar approach could be applied to the treatment of selected malignancies in other portions of the body. Much basic and clinical research remains to be done to determine the utility of this novel local treatment modality.

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References

- Ali-Osman F, Giblin J, Dougherty D, et al: Application of *in vivo* and *in vitro* pharmacokinetics for physiologically relevant drug exposure in a human tumor clonogenic cell assay. *Cancer Res* 47:3718-3724, 1987
- Blasberg RG, Groothuis DR: Chemotherapy of brain tumors: physiological and pharmacokinetic considerations. *Semin Oncol* 13:70-82, 1986
- Bouvier G, Penn RD, Kroin JS, et al: Direct delivery of medication into a brain tumor through multiple chronically implanted catheters. *Neurosurgery* 20:286-291, 1987
- Brem H: Polymers to treat brain tumors. *Biomaterials* 11:699-710, 1990
- Brem H, Kader A, Epstein JI, et al: Biocompatibility of biodegradable, controlled-release polymer in the rabbit brain. *Select Cancer Ther* 5:55-65, 1989
- Brem H, Mahaley MS Jr, Vick NA, et al: Interstitial chemotherapy with drug polymer implants for the treatment of recurrent gliomas. *J Neurosurg* 74:441-446, 1991
- Brem H, Tamargo RJ, Olivi A: Delivery of drugs to the brain by use of a sustained-release polyanhydride polymer system. *J Appl Toxicol* (In press, 1992)
- Brown LR, Wei CL, Langer R: *In vivo* and *in vitro* release of macromolecules from polymeric drug delivery systems. *J Pharmacol Sci* 72:1181-1185, 1983
- Cascino TL, Byrne TN, Deck MD, et al: Intra-arterial BCNU in the treatment of metastatic brain tumors. *J Neurooncol* 1:211-218, 1983
- Chasin M, Domb A, Ron E, et al: Polyanhydrides as drug delivery systems, in Langer R, Chasin M (eds): *Biodegradable Polymers as Drug Delivery Systems*. New York: Marcel Dekker, 1990, pp 43-70
- Chasin M, Lewis D, Langer R: Polyanhydrides for controlled drug delivery. *Biopharm Manufact* 1:33-46, 1988
- Diemath HE: Lokale Anwendung von Zytostatika nach

Biodegradable polymers

- Exstirpation von Glioblastomen. *Wien Klin Wochenschr* **99**:674-676, 1987
13. Fu YA, Shihab Z, Chen TT, et al: Clinical evaluation of the OCUSERT-pilocarpine system: a five year follow-up report. *Glaucoma* **3**:31-35, 1981
 14. Garfield J, Dyan AD: Postoperative intracavitary chemotherapy of malignant gliomas. A preliminary study using methotrexate. *J Neurosurg* **39**:315-322, 1973
 15. Girgis M, Shih-Chang W: *Stereotactic Atlas of the Rabbit Brain*. St Louis: WH Green, 1981
 16. Goodson JM, Holborow D, Dunn RL, et al: Monolithic tetracycline-containing fibers for controlled delivery to periodontal pockets. *J Periodontol* **54**:575-579, 1983
 17. Goodson JM, Offenbacher S, Farr DH, et al: Periodontal disease treatment by local drug delivery. *J Periodontol* **56**:265-272, 1985
 18. Greenberg HS, Ensminger WD, Chandler WF, et al: Intra-arterial BCNU chemotherapy for treatment of malignant gliomas of the central nervous system. *J Neurosurg* **61**: 423-429, 1984
 19. Gutin PH, Leibel SA, Wara WM, et al: Recurrent malignant gliomas: survival following interstitial brachytherapy with high-activity iodine-125 sources. *J Neurosurg* **67**: 864-873, 1987
 20. Heller J: Biodegradable polymers in controlled drug delivery. *Crit Rev Ther Drug Carrier Sys* **1**:39-90, 1984
 21. Hochberg FH, Parker LM, Takvorian T, et al: High-dose BCNU with autologous bone marrow rescue for recurrent glioblastoma multiforme. *J Neurosurg* **54**:455-460, 1981
 22. Hochberg FH, Pruitt A: Assumptions in the radiotherapy of glioblastoma. *Neurology* **30**:907-911, 1980
 23. Hochberg FH, Pruitt AA, Beck DO, et al: The rationale and methodology for intra-arterial chemotherapy with BCNU as treatment for glioblastoma, in Howell LS (ed): *Intra-Arterial and Intracavitary Chemotherapy*. Boston: Martinus Nijhoff, 1984, pp 97-109
 24. Kelly PJ, Daumas-Duport C, Scheithauer BW, et al: Stereotactic histologic correlations of computed tomography- and magnetic resonance imaging-defined abnormalities in patients with glial neoplasms. *Mayo Clin Proc* **62**:450-459, 1987
 25. Kornblith PL, Walker M: Chemotherapy for malignant gliomas. *J Neurosurg* **68**:1-17, 1988
 26. Kroin JS, Penn RD: Intracerebral chemotherapy: chronic microinfusion of cisplatin. *Neurosurgery* **10**:349-354, 1982
 27. Laurencin CT, Domb AJ, Morris CD, et al: High dosage administration of polyanhydrides *in vivo*: studies of biocompatibility and toxicology. *Proc Int Symp Control Rel Bioact Mater* **14**:140, 1987 (Abstract)
 28. Leibel SA, Sheline GE: Radiation therapy for neoplasms of the brain. *J Neurosurg* **66**:1-22, 1987
 29. Leong KW, Brott BC, Langer R: Biodegradable polyanhydrides as drug-carrier matrices. I: Characterization, degradation, and release characteristics. *J Biomed Mater Res* **19**:941-955, 1985
 30. Madajewicz JS, West CR, Park HC, et al: Phase II study — intra-arterial BCNU therapy for metastatic brain tumors. *Cancer* **47**:653-657, 1981
 31. Mahaley MS, Mettlin C, Natarajan N, et al: National survey of patterns of care for brain-tumor patients. *J Neurosurg* **71**:826-836, 1989
 32. Neuweit EA, Frenkel EP, Gumerlock MK, et al: Developments in the diagnosis and treatment of primary CNS lymphoma. *Cancer* **58**:1609-1620, 1986
 33. Patchell RA, Tibbs PA, Walsh JW, et al: A randomized trial of surgery in the treatment of single metastases to the brain. *N Engl J Med* **322**:494-500, 1990
 34. Pitt CG, Schingler A: The design of controlled drug delivery systems based on biodegradable polymers, in Hafez ESE, van Os WAA (eds): *Biodegradable Delivery Systems for Contraceptives*. Lancaster, England: MTP Press Ltd, 1980, Vol 1, pp 17-46
 35. Prados M, Leibel S, Barnett CM, et al: Interstitial brachytherapy for metastatic brain tumors. *Cancer* **63**:657-660, 1989
 36. Rall DP, Zubrod CG: Mechanism of drug absorption and excretion. Passage of drugs in and out of the central nervous system. *Annu Rev Pharmacol* **2**:109-128, 1962
 37. Rubin RC, Ommaya AK, Henderson ES, et al: Cerebrospinal fluid perfusion for central nervous system neoplasms. *Neurology* **16**:680-692, 1966
 38. Shapiro WR: Introduction: brain tumors. *Semin Oncol* **13**:1-3, 1986
 39. Shapiro WR, Green SB, Burger PC, et al: A randomized comparison of intra-arterial vs. intravenous BCNU for patients with malignant glioma: interim analysis demonstrating lack of efficacy for IA BCNU. *Proc Am Soc Clin Oncol* **6**:69, 1987 (Abstract)
 40. Solero CL, Monfardini S, Brambilla C, et al: Controlled study with BCNU vs CCNU as adjuvant chemotherapy following surgery plus radiotherapy for glioblastoma multiforme. *Cancer Clin Trials* **2**:43-48, 1979
 41. Soskolne A, Golomb G, Friedman M, et al: New sustained release dosage form of chlorhexidine for dental use. II. Use in periodontal therapy. *J Periodontal Res* **18**: 330-336, 1983
 42. Stewart DJ, Benjamin RS, Zimmerman S, et al: Clinical pharmacology of intraarterial cis-diamminedichloroplatinum (II). *Cancer Res* **42**:2059-2062, 1982
 43. Tamargo RJ, Epstein JI, Reinhard CS, et al: Brain biocompatibility of a biodegradable, controlled-release polymer in rats. *J Biomed Mater Res* **23**:253-266, 1989
 44. Tamargo RJ, Epstein JI, Yang MB, et al: Interstitial vs systemic chemotherapy of the intracranial 9L gliosarcoma: controlled-release polymers for local therapy. *J Neurosurg* **70**:311A, 1989
 45. Walker MD, Alexander E Jr, Hunt WE, et al: Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. *J Neurosurg* **49**:333-343, 1978
 46. Walker MD, Green SB, Byar DP, et al: Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. *N Engl J Med* **303**:1323-1329, 1980
 47. Wara WM: Radiation therapy for brain tumors. *Cancer* **55**:2291-2295, 1985
 48. Weiss SR, Raskind R: Treatment of malignant brain tumors by local methotrexate. A preliminary report. *Int Surg* **51**:149-155, 1969
 49. Yang MB, Tamargo RJ, Brem H: Controlled delivery of 1,3-bis(2-chloroethyl)-1-nitrosourea from ethylene-vinyl acetate copolymer. *Cancer Res* **49**:5103-5107, 1989

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Address reprint requests to: Stuart A. Grossman, M.D., Department of Neuro-Oncology, The Johns Hopkins Oncology Center, 600 North Wolfe Street, Baltimore, Maryland 21205.