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INTERSTITIAL DRUG THERAPY FOR BRAIN TUMORS: A CASE STUDY

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ABSTRACT

A novel method of delivering cancer chemotherapeutic drugs to the brain in concentrations higher than those achievable by standard routes of administration has been developed. The drug was incorporated into wafers (trademarked BIODEL®) of a biodegradable, biocompatible polymer. The drug chosen for initial studies was BCNU (carmustine, 1,3-bis[2-chloroethyl]-1-nitrosourea), the most widely used chemotherapeutic agent for the disease to be treated, glioblastoma multiforme, a universally fatal form of brain cancer. Preclinical studies in a number of species demonstrated the safety of this material implanted either directly into the brain or subcutaneously. Efficacy studies in rats demonstrated that implanting BIODEL® wafers containing BCNU either at the time of tumor implantation or after 4 days of tumor growth could significantly prolong survival of the animals, while greatly diminishing side effects of the BCNU therapy.

The safety of this material implanted into these patients during a Phase I/II study has been demonstrated. No systemic side effects of doses of BCNU which would produce marked effects on the hemopoeitic system when injected intravenously have been observed. Further studies,

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designed to measure the efficacy of this approach to the treatment of brain cancer in a multicentered Phase III clinical trial in the United States and Canada, are currently underway.

INTRODUCTION

The chemotherapeutic treatment of brain tumors has historically not been marked by great success. One reason for this marked lack of efficacy is the blood-brain barrier, preventing or limiting the access of systemically administered chemotherapeutic agents to the tumor. Local, controlled delivery of such drugs directly to the tumor site bypasses this barrier, and has the potential both to increase local drug concentrations, and thereby, efficacy, and to decrease the systemic toxicity of these agents. The polyanhydride polymers offer a biocompatible vehicle for such drug delivery, and we are currently utilizing one of these to deliver BCNU directly upon the resected surface of tumor remaining after surgery.

A depot formulation is a drug reservoir with a mechanism for controlled release of drug. In order to gain access to local tissues, the depot must be implanted. Once implanted, the depot releases drug until the reservoir is depleted, at which time the depot would typically need to be refilled or removed. Biodegradable materials avoid the necessity of removal and are thus advantageous for some specific applications.

Biodegradable, controlled-release, polymeric delivery systems enjoy a number of advantages over other depot delivery systems in terms of simplicity of design and predictability of release. These features are dependent upon release that is controlled solely by degradation of the polymer matrix. In many cases using previously available polymers, the matrix is hydrophilic, and water absorption into the polymeric matrix itself promotes hydrolytic degradation of the implant in the interior of the polymer matrix. This process leads to "burst release" or "drug dumping", which is undesirable for such agents as cancer chemotherapeutic drugs, since with such highly toxic molecules, the results of severe drug dumping might be fatal. To maximize control over the release process, it is desirable to have a polymeric system which degrades only through surface erosion and deters the permeation of water into the matrix. This offers the additional advantage of protecting the drug from degradation by the biological fluids of the body, especially for drugs with short biological half-lives, such as BCNU, whose half-life in biological fluids is approximately 12 minutes.

In order to maintain biodegradability and prevent permeation of water, certain characteristics of the polymer must be established. The ideal polymer would have a hydrophobic backbone, but with a water labile linkage. In designing a biodegradable system that would erode in a controlled homogenous manner, without requiring additives, a number of polymer systems

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have been investigated. The high lability of the anhydride linkage makes the polyanhydrides suitable candidates for such development.

The discussion below briefly describes formulation, production, stability, sterilization, safety and efficacy experiments which have been performed[1] on the prototypical polyanhydride for the delivery of BCNU directly onto the cut surface of the brain following surgical excision of a Grade III or IV anaplastic astrocytoma.

Through our work on the BCNU-polymer system, we have learned a great deal about the requirements for such drug delivery systems. It is very important that the materials which are to be used in the preclinical animal studies are as much like the ultimate clinical implants as is possible. Confirmation of parameters like content uniformity, in vitro release rates of drugs, molecular weight of the polymers utilized, reproducibility of the ratio of monomers in the copolymers from polymer batch to batch, sterility, lack of pyrogenicity, porosity, and many others, are very important in being able to predict human results from those observed either in animal models or from *in vitro* studies. It is therefore critical that these parameters be optimized and that the process used for the production of the preclinical supplies be reproducible and identical to that which will be used for the human studies.

RESULTS

Description of BCNU-Polyanhydride Wafer Formulation

The experimental formulation currently undergoing clinical trials is an implant (wafer) made of biodegradable poly[bis(p-carboxyphenoxy)propane:sebacic acid] anhydride in a ratio of 20:80, in which the active component BCNU is homogeneously dissolved. The individual wafers have diameters of 1.4 cm, are 1 mm thick, and weigh 200 mg.

The polyanhydride, which functions as the excipient for release of BCNU, is a random copolymer of sebacic acid and bis(p-carboxyphenoxy)propane. The weight-average molecular weight of the polymer is approximately 30,000-80,000.

Content Uniformity

One of the key features required of such a delivery system is the uniform distribution of drug throughout the implant. A study was performed to determine the content uniformity of pieces of wafers made 1) by dry mixing and pressing or 2) by producing a solid solution of drug in the polymer. The dry mixture was prepared by triturating BCNU and the powdered polymer

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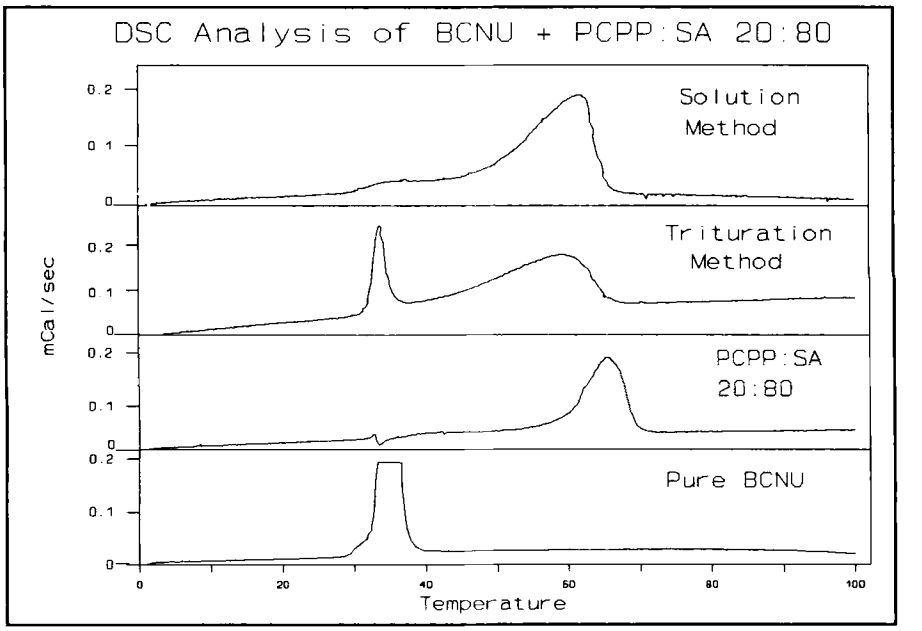

Figure 1. Differential scanning calorimetry scans of samples of PCPP:SA 20:80 with or without 10% BCNU.

and pressing the resulting mixture in a Carver press. The solid solution was produced by co-dissolving the BCNU and polymer in methylene chloride, spray-drying this solution, and pressing the resulting powder in a similar fashion. Small pieces of each of these types of wafers were then assayed for BCNU content to determine content uniformity within the wafers. The dry mixture average BCNU content was 9.82% with a standard deviation of 1.14, whereas the average of the solution process was 10.40% with a standard deviation of 0.17. Represented as a percent standard deviation (the standard deviation divided by the mean value), the dry mixture showed 11.6% variation, while the solution process showed only 1.6% variation, demonstrating a marked improvement in the content uniformity of the solid solution wafers.

That the BCNU existed as a solid solution, dissolved in the polymer, was shown by DSC analysis (Figure 1). BCNU demonstrated a single melting point at approximately 32°C, and the pure polymer showed a broad band beginning at approximately 35°C, corresponding to the glass transition, and a more narrow band corresponding to the melting point of the polymer, at approximately 65°C. For a dry mixed wafer of BCNU and polymer, a distinct peak was seen for both BCNU and the polymer, although the polymer melting peak occurred at a lower tem-

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perature and was broader than the corresponding peak for pure polymer, due to the formation of a small amount of BCNU actually dissolved in the polymer as a solid solution. For material made by codissolving the polymer and BCNU together to produce a solid solution, no peak of BCNU was observed, demonstrating that all of the BCNU was present as a solid solution. Note that the absence of a reaction between the polymer and BCNU was demonstrated by dissolving this material in methylene chloride and assaying for unchanged BCNU by HPLC. Quantitative recovery of the BCNU was observed.

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Stability

A stability study has been performed using wafers containing either 1% or 10% BCNU by weight, which was designed to determine the stability of the BCNU in the polymeric matrix, and which continued for 1 year. In this study, wafers of both BCNU concentrations were produced, packaged, and sterilized by gamma irradiation using 2.5 Mrad. These materials were then placed on stability at either -20°C or 5°C, and samples were removed at predefined intervals over one year. At the appropriate times, the wafers which were removed from the stability cabinets were dissolved in methylene chloride, and assayed for BCNU content by HPLC analysis. HPLC assays utilized the Hewlett Packard Model 1084B System with the fixed wavelength detector (254 nm). A Waters 30 cm μPorasil Column (P/N 27477) was used with recently degassed methylene chloride as the mobile phase, and the system was stabilized at a flow rate of 1 mL/min at a solvent and column temperature of 30°C. There was no appreciable change in the concentration of BCNU measured in these wafers under either storage condition.

The stability of PCPP:SA, 20:80, to gamma irradiation for sterilization was also determined. The samples were maintained either at ambient, room temperature conditions, or on dry ice, during the irradiation. Although small changes were seen in the molecular weights of the polymers under either of these conditions, neither was deemed to be unacceptable. In addition, neither the T_m nor the T_g were affected by the irradiation, and the infrared spectra remained unchanged, as well. It was concluded that gamma irradiation does not markedly effect the polymer. For routine sterilization, 2.5 Mrad is used with the samples maintained on dry ice throughout the procedure.

The stability of BCNU to gamma irradiation was also determined. The samples were sterilized at either ambient temperature or on dry ice, with three different radiation dosages

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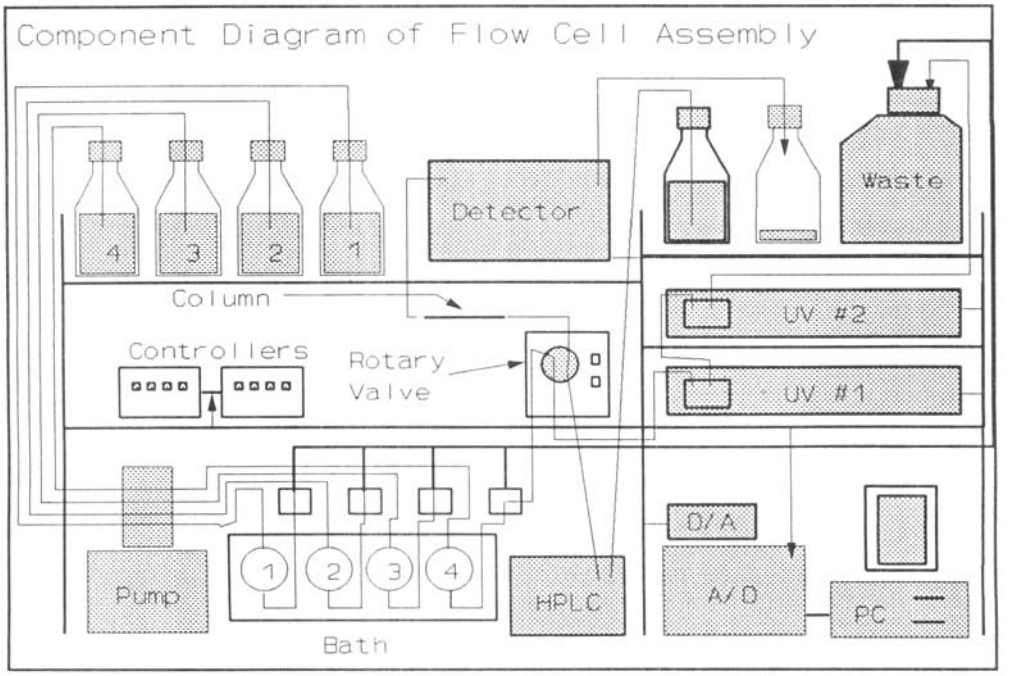

Figure 2. Diagrammatic representation of the flow-cell system used for the analysis of wafers containing PCPP:SA 20:80 and BCNU.

(1.5, 2.0, and 2.5 Mrads). HPLC analysis and melting point measurement of these samples did not show any drug degradation.

Development of Assays for Release Kinetics

The development of dosage forms which reproducibly deliver specific drugs over prolonged periods of time was critical for the success of this project. Crucial to this development is the ability to accurately measure the release kinetics of drugs incorporated into these materials. During the development of the BCNU wafers, a flow-through system has been developed for this purpose, and is diagrammatically represented in Figure 2. Buffer is pumped from four storage containers through four flow cells. The effluent from each cell is controlled by a computer-controlled rotary valve which can direct the flow either through one or both ultraviolet flow-through cells, through an HPLC column, or to waste. Data reduction using an analog-to-

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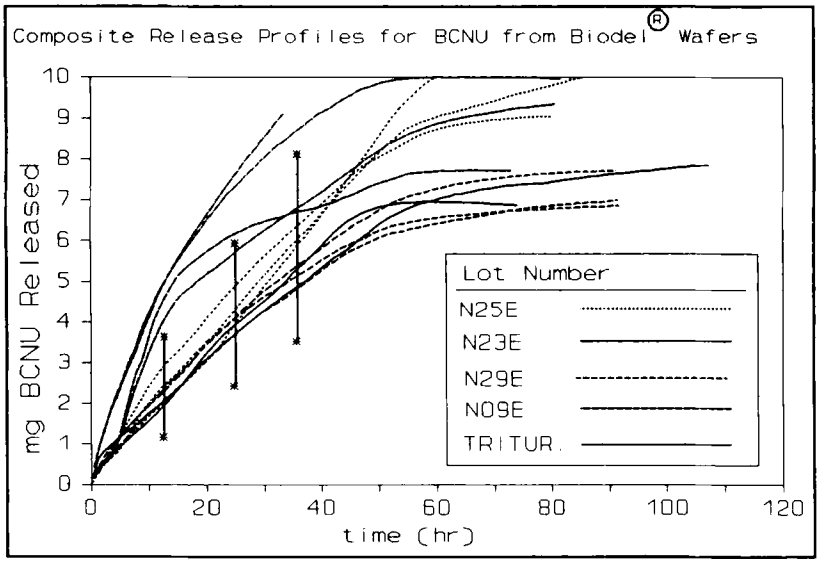

Figure 3. Release of BCNU from various batches of wafers. The vertical bars represent the acceptance specifications for this dosage form.

digital converter is accomplished through a computer interface to IBM-compatible microcomputers. Two such sets of equipment, capable of determining release and degradation kinetic profiles on eight different samples simultaneously, are available.

Chemistry and Quality Controls

For the BCNU project, purification schemes, quality control procedures, detailed specifications and acceptance criteria had to be established for the CPP and SA monomers, both prepolymers, and the PCPP:SA 20:80 polymer[1]. These involved chemical synthesis and scaleup expertise and extensive analytical chemistry support. In particular, it was found that the purity of the monomers and prepolymers played a critical role in the quality of the final polymer, affecting both the biocompatibility and rate of degradation of the final polymer. It was found that to properly control the production of this polymeric delivery system, the sensitive *in vitro* assay described above for measuring the release rate of BCNU had to be developed.

Having determined the *in vitro* release profile of BCNU from a series of batches of BIODEL® wafers, specifications for this *in vitro* release assay were set, and are represented by

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the three vertical bars in Figure 3. The specifications were set such that every batch had to fall within all three of the windows represented by these bars. The release profiles of three such batches, N25E, N23E, and N29E, are shown. Also shown in this figure is the release profile of wafers produced by trituration, as described above; these wafers released BCNU considerably faster than did the solid solution wafers. Finally, the release profile of a batch which did not meet the release specification, N09E, and which also failed other acceptance assays, is also shown.

Determination of Potential Drug - Polymer Interactions

Since the polyanhydrides have the potential for chemical interaction with several functional groups normally found on many drugs[2,3], it is critical to determine whether such interactions occur for each drug incorporated using each specific fabrication technique. It has been shown that the potential for such interactions is higher at elevated temperatures[2], so methods such as hot-melt microsphere production, or injection molding, might be expected to have the highest probability of such interactions.

A typical experiment to determine whether such potential interaction occurs is as follows. First, spectroscopic analysis of the drug-polymer matrix can sometimes demonstrate the presence of bonds which would not be present in unreacted materials. For instance, amines can form amide bonds with polyanhydrides, which can be detected by absorbance in the region of 1630-1700 cm^{-1} using IR spectrophotometry.

A more sensitive method involves direct assay of unchanged (or, conversely, reacted) drug. The final fabricated material is dissolved in methylene chloride. For drugs such as BCNU which are soluble in methylene chloride, it is possible to directly assay this solution for unchanged drug using typical analytical techniques, such as HPLC. Using both methods for determining an interaction between polymer and BCNU, no such interactions have been observed.

The difference in rates of release of BCNU from wafers produced by the trituration or solution methods discussed above is also seen *in vivo*[4,5], as shown in Figure 4. Wafers of PCPP:SA 20:80 containing tritiated BCNU were prepared by either the solution or trituration methods, as described above, and were implanted into the brains of rabbits. The animals were sacrificed at various times after implantation, and the brains were removed, fixed, and processed for quantitative autoradiography. To quantitate the percentage of the brain exposed to BCNU released from these wafers, the following calculation was performed. The percentage of the brain in which the radioactivity from the tritiated BCNU released from the wafers exceeded the

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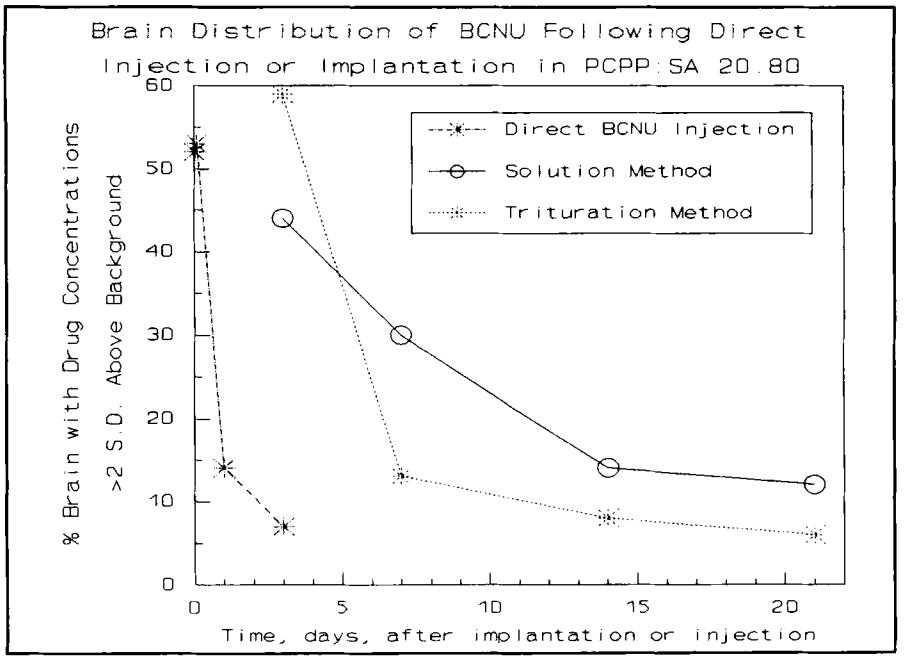

Figure 4. *In vivo* release of tritiated BCNU in rabbit brain from a matrix of PCPP:SA 20:80.

background counts by at least two standard deviation units was plotted as a function of time following implantation. A control set of rabbits had a solution of BCNU injected directly into the same location in the brain as the implants. The rate of release of BCNU from wafers prepared by the solution method was slower than that from wafers prepared by the trituration method, in agreement with the *in vitro* results. At the longest time measured, 21 days after implantation, these animals had significant levels of BCNU in an area of brain almost twice as large as those receiving the wafers produced by the trituration method.

This figure also shows that the use of this polymeric delivery system, also known as the BIODEL® polymer, for BCNU greatly increases the time over which the brains of these animals are exposed to significant BCNU concentrations. The brains of animals which received a single injection of the same amount of BCNU contained in the wafers were almost free of BCNU by three days after injection. Since it has been shown conclusively both *in vitro* and *in vivo* that the key determinant of the ability of a drug to kill cancer cells depends on the product of the drug's concentration and the time over which the drug and the cancer cells are in

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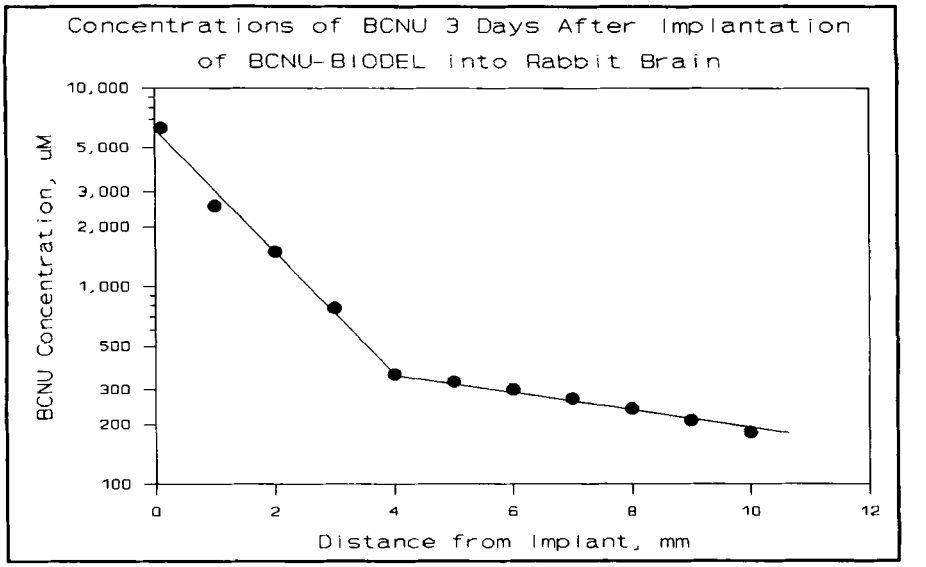

Figure 5. Concentrations of BCNU at various distances from the implantation site in rabbit brain at three days following implantation.

contact[16,17], it seems reasonable to conclude that the greatly increased time over which BCNU is delivered to the brain using these BIODEL® wafers should increase the efficacy of BCNU.

Since these studies utilized autoradiographic techniques, it was important to determine the chemical nature of the material measured using radiochemical procedures. Using punch biopsies of the brain slices which were measured autoradiographically, it was shown using thin layer chromatography that about 30% of the radioactivity was associated with unchanged BCNU[5]. It is therefore concluded that the measurements described above accurately reflect brain concentrations of BCNU.

From these same autoradiography studies, it is also possible to determine the local brain concentrations of BCNU which can be achieved using the BIODEL® delivery system. The brain adjacent to the surface of these wafers (signified by zero on the distance axis in this figure) is exposed to concentrations of BCNU of approximately 6.5 mM at three days following implantation. Even as far as 10 mm from this surface, the local concentrations of BCNU are approximately 200 μM. This concentration range is certainly much higher than the brain concentrations achieved through a single intravenous administration of BCNU, which is the standard treatment for this disease.

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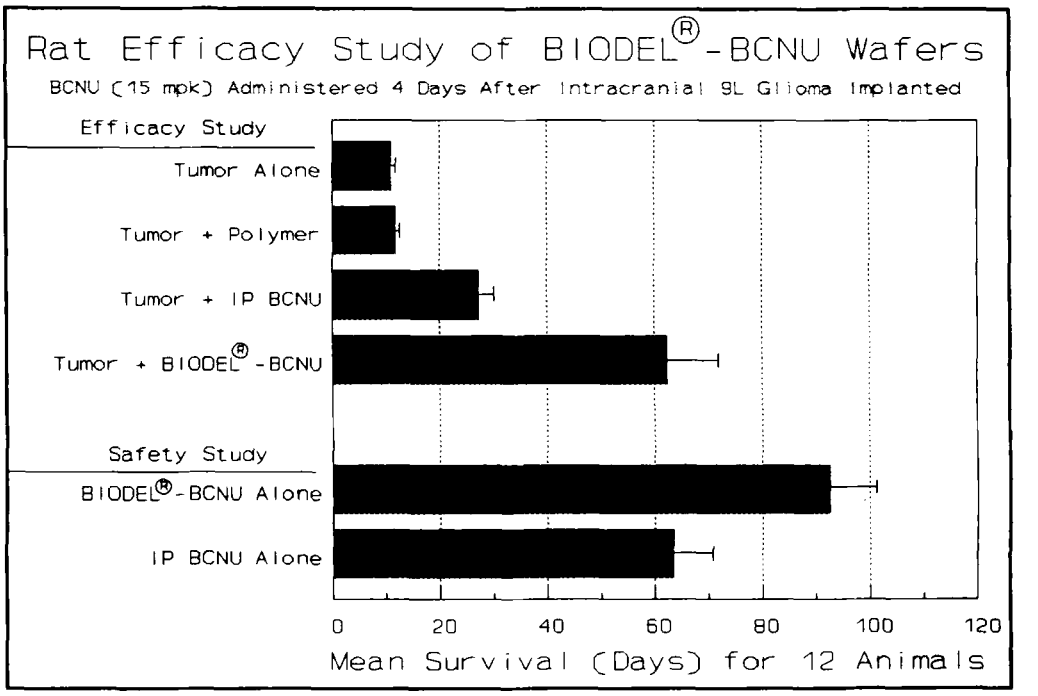

Figure 6. Mean survival of rats following various treatment regimens. Any animals surviving to 120 days post-treatment were sacrificed at that time.

Safety Evaluation

A series of biocompatibility studies has been performed on several polyanhydrides. As evaluated by mutation assays[2], the degradation products of the polymer were non-mutagenic and non-cytotoxic. *In vitro* tests measuring teratogenic potential were also negative. Growth of types of mammalian cells in tissue culture was also not affected by these polymers[2]; both the cellular doubling time and cellular morphology were unchanged when either bovine aorta endothelial cells or smooth muscle cells were grown directly on the polymeric substrate.

Implants in the rabbit corneas exhibited no observable inflammatory characteristics over a period of six weeks. Compared to other previously tested polymers, the inertness of these polyanhydrides rivals that of the biocompatible poly(hydroxyethyl methacrylate) and ethylene-vinyl acetate copolymer. Histological examination of the removed corneas also revealed the absence of inflammatory cells[2].

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Additional evidence of biocompatibility was provided from subcutaneous implantation tests in rats[2,4,6]. After a period of six months, only very slight tissue encapsulation was seen around implantations of pure PCPP. After 56 days, no tissue encapsulation was seen around implants of PCPP:SA 20:80. No inflammation was apparent in the tissues adjacent to the implant from histological evaluations of PCPP, and only very slight inflammation was seen following implantation of PCPP:SA 20:80. No changes were seen in measures of blood chemistry and hematology of the rats during the degradation of these polymers *in vivo*, and gross and microscopic postmortem analysis also did not reveal any abnormalities[2,7,8].

The biocompatibility of the PCPP:SA 20:80 with rat brain was studied to evaluate the safety of the polymer and its degradation products in the rat, and to compare it to two standard materials which have been extensively studied and proven to be nontoxic or mildly inflammatory to neural tissue[9,10]. These materials are Gelfoam (absorbable gelatin sponge) and Surgicel (another commonly used material in brain surgery). The animals were sacrificed using CO_2 asphyxiation, one group per day on days 3, 6, 10, 15, 21, 28, and 36 following surgery. Histological evaluation of the tissue demonstrated a small rim of necrosis around the implant, and a mild to marked cellular inflammatory reaction limited to the area immediately adjacent to the polymer implant site, slightly more marked than Surgicel at the earlier time points, but noticeably less marked than Surgicel at the later times. The reaction to Gelfoam was essentially equivalent to sham-operated control animals. Using PCPP:SA 50:50 in rabbit brain, even less of an inflammatory reactions was observed; the polymer was essentially equivalent to Gelfoam[11].

The biocompatibility of the PCPP:SA 20:80 with the monkey brain was studied to measure the direct effects of the polymer, polymer degradation products, and polymer containing BCNU on the monkey brain[12,13], the best experimental model of the human brain.

Blood samples were periodically obtained from each monkey for blood chemistry and hematology analyses. On the 9th day following surgery, the animals had both non-contrast and contrast enhanced CT studies performed. On the 12th day following surgery, MRI studies were performed. At the conclusion of the experiment, samples of the brain and 35 other tissues were prepared for histological examination.

No abnormalities were noted in any of the computer-assisted tomography or magnetic resonance imaging scans, nor in the blood chemistry or hematology evaluations. No systemic effects of the implants were noted on histological examination of any of the tissues examined. No unexpected or untoward reactions to the treatments were observed.

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Efficacy Studies

Several different efficacy studies were performed in rats[14,15]. The first[14] involved simultaneous implantation of the 9L glioma and BIODEL® wafers containing BCNU. Although prolongation of life was observed, this experimental design can be criticized as measuring an effect on tumor implantation, rather than on effecting a pre-existing tumor. For that reason, a second study was performed[15], in which the tumor was implanted, and four days later, the experimental therapy was begun. Several groups of animals were incorporated into the study design, including no further therapy, sham surgery, intraperitoneal injection of 15 mpk BCNU, and implantation of either BCNU-containing or placebo BIODEL® wafers. All animals surviving to 120 days were sacrificed.

As can be seen, although intraperitoneal BCNU slightly increased survival, it did so only at a toxic dose, shown in the bottom section of the figure. Unloaded polymer had no effect on survival, but BIODEL® wafers containing BCNU dramatically increased the mean survival, while at the same time decreasing the toxicity of BCNU substantially.

Furthermore, when the data were examined using a Kaplan-Meier survival analysis, as shown in Figure 7, about 30% of the animals treated with BIODEL® wafers containing BCNU survived throughout the study, and at necropsy were found to have no residual tumor. These animals can be considered cured. It was concluded that delivery of BCNU directly to the site of the tumor dramatically increased its efficacy, while at the same time decreased its toxicity substantially.

Based on all of the physical, chemical, safety and efficacy data collected during the preclinical studies, it was concluded that the project should proceed to clinical studies.

Clinical Studies

BIODEL® wafers with the cancer chemotherapeutic agent BCNU incorporated into them, have been studied in man for the treatment of glioblastoma multiforme, a universally fatal form of brain cancer. In these studies, patients undergoing reoperation for the removal of the bulk of the tumor have had the surgical cavity lined with the polymeric drug delivery system containing BCNU. Following surgery, the BCNU is then released directly onto adjoining cancer cells that may not have been removed during surgery.

The first clinical study[18], designed to measure the safety of this approach, and conducted at five major hospitals across the United States, was an open-label ascending dose tolerance study in recurrent Grade III or IV anaplastic astrocytoma patients. At the time of reoperation,

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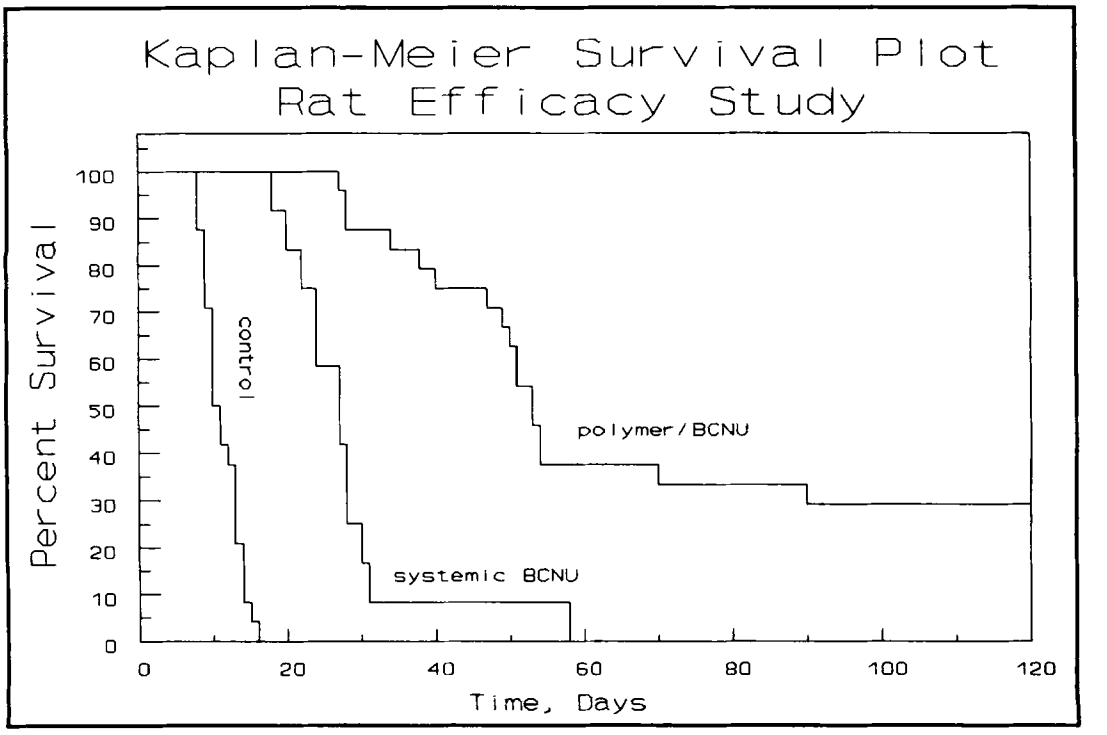

Figure 7. Kaplan-Meier survival curves for the groups described in Figure 6.

up to eight 1 mm thick wafers, 1.4 cm in diameter, were placed to line the surgical cavity where remaining tumor was suspected. Three dosage groups were studied; i.e., wafers made of polymer containing three different concentrations of BCNU were used in three groups of patients. Safety evaluations of these patients demonstrated the safety of this approach, since no systemic or local toxic manifestations of the treatment were observed. The next clinical study, a double-blind, placebo controlled study designed to determine efficacy, also involving patients with recurrent Grade III or IV anaplastic astrocytoma, began in 1989, and will involve hundreds of patients at over 15 centers in the United States and Canada.

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