

Biocompatibility of a Biodegradable, Controlled-Release Polymer in the Rabbit Brain

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ABSTRACT

The biodegradable polyanhydrides are a new class of controlled release polymers developed for the interstitial delivery of drugs to their target site in the brain or other organs over periods ranging from days to years. These polymers can release molecules of any size in a predictable fashion. Their degradation products are non-cytotoxic and biocompatible. The biocompatibility of a biodegradable polyanhydride, the copolymer of poly[bis(p-carboxyphenoxy)propane] anhydride and sebacic acid (PCPP-SA) in a 50:50 formulation, was studied in the rabbit brain. Twenty adult New Zealand White male rabbits underwent implantation of PCPP-SA in a frontal lobe and absorbable gelatin sponge (Gelfoam) in the other frontal lobe. The animals were evaluated daily until the time of sacrifice. Groups of four animals were sacrificed sequentially on post-operative days 1, 3, 7, 21, and 60, and the brains processed for histological evaluation. None of the animals showed behavioral changes or neurological deficits suggestive of toxicity and all that received implants survived to their date of sacrifice. The histological examination showed no significant differences between the tissue reaction from PCPP-SA compared to Gelfoam. The polymers were also tested in the rabbit cornea bioassay and did not induce an inflammatory response. We conclude that PCPP-SA (50:50), a new biodegradable polymeric matrix that can be surgically implanted for the interstitial delivery of drugs in the brain, is biocompatible in the rabbit brain.

INTRODUCTION

The localized, sustained administration of biologically active agents using polymeric delivery systems is an alternative to the systemic administration of drugs (8,11). A new class of

Polymeric delivery devices, the biodegradable polyanhydrides (12,13,14) has been developed for interstitial drug delivery in the brain. This polymer has excellent release kinetics governed by the surface erosion of the matrix (12). Thus, as the polymer degrades, the molecules stored within it are released. Furthermore, the degradation products of the polyanhydride have been shown to be non-mutagenic, non-cytotoxic, and non-teratogenic (13). The biocompatibility of the biodegradable polyanhydride has been confirmed *in vivo* by implantation in the rabbit cornea and in the rat subcutaneous tissue, and *in vitro* using bovine aortic endothelial cells and smooth muscle cells (13).

A biodegradable polymeric delivery system may have numerous applications in neurological surgery, where the presence of the blood-brain barrier limits pharmacological access to the central nervous system, and the systemic administration of drugs destined to reach the central nervous system may carry significant and unwanted generalized side effects. We therefore determined the biocompatibility of PCPP-SA implanted in the rabbit brain by comparing it with absorbable gelatin sponge (Gelfoam), which is used routinely in neurosurgery and has been shown to be non-toxic to neural tissue (1). The inflammatory potential of the polymer was also tested in the rabbit cornea bioassay, a highly sensitive biocompatibility assay that has been previously used to grade the inflammatory nature of polymeric delivery systems (10).

MATERIALS and METHODS

Polymer Preparation

The biodegradable polyanhydride poly[bis(p-carboxyphenoxy)propane-sebacic acid anhydride] (PCPP-SA) in a 50:50 ratio was synthesized by melt polycondensation as previously described (12). The polymer mixture was formulated into disks measuring 3 mm in diameter and 1-2 mm in height by compression molding (12). Using the surgical microscope, the disks were cut into pieces 2 x 2 x 2 mm³ for implantation. The implants were sterilized by UV irradiation for 1 hour.

Animals

New Zealand White rabbits weighing about 1.5-2.5 kg were obtained from Bunnyville Farm (Littlestown, Pennsylvania). They were kept in standard animal facilities, one animal per cage, and given free access to food and water.

Anesthesia

The animals were anesthetized with a mixture of xylazine 15-17 mg/kg and ketamine 15-17 mg/kg injected intramuscularly for the intracranial and corneal implantations as well as for the stereomicroscopic examinations of the cornea.

Brain Implantation

The head of the animal was shaved and prepared with 70% ethyl alcohol and Prepodyne solution. A 3 cm incision was made in the midline and the subcutaneous tissues and periosteum removed from the skull by blunt dissection. Bilateral burr holes 5 mm in diameter were made with their center 2 mm anterior to the coronal suture and 6 mm lateral to the sagittal suture. A No. 11 blade

was used to pierce the dura and the underlying cortex to a depth of about 3 mm. The bleeding was allowed to subside spontaneously. A piece of the biodegradable polyanhydride was inserted into one hemisphere and a piece of Gelfoam (Upjohn Company, Kalamazoo, Michigan) of similar volume was inserted into the other. The skin incision was closed with clips.

Rabbit Cornea Bioassay

The polymer was implanted into four rabbit corneas using a modification of the technique of Gimbrone et al. (6). The cornea was irrigated with proparacaine hydrochloride 0.5% (Allergan Pharmaceuticals, Inc., Irvine, California) for local anesthesia. The globe of the anesthetized animal was gently proptosed and secured in this position with a latex dental dam (Hygenic Corp., Akron, Ohio). Using a Bard-Parker No. 11 blade, a 2 mm superficial incision, approximately 0.1 mm deep, was made on the cornea below its central region. An iris spatula was inserted into the corneal incision and advanced within the corneal stroma toward the limbus, stopping at 2 mm of the corneoscleral junction, thus creating a pocket within the cornea. Pieces of polymer measuring 1.0 X 1.0 X 0.5 mm³ were introduced into the pocket. The dental dam was removed, the globe repositioned within the orbit, and the cornea irrigated again with proparacaine hydrochloride 0.5%. The corneas were examined twice weekly for three weeks with a Zeiss Slit Lamp Stereomicroscope (Carl Zeiss, Inc., Thornwood, New York) looking for corneal edema or neovascularization as evidence of an inflammatory reaction.

Postoperative Evaluation

The animals with brain implants were evaluated on a daily basis after surgery. Particular attention was given to (i) behavioral changes such as decreased alertness, passivity, impaired grooming, restlessness, irritability, and fearfulness, and to (ii) focal motor neurological deficits (4).

Histology

The animals were sacrificed by the intravenous administration of an overdose of thiamylal sodium. The brains were removed and placed in 10% phosphate buffered formalin for 7-10 days, then cut, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. A qualitative assessment was made of the extent of gliosis and inflammation in the region of the implants.

RESULTS

Twenty rabbits underwent implantations and were sequentially sacrificed. One rabbit was excluded from the study when noted to have had diarrhea preoperatively. Three rabbits were sacrificed on the first postoperative day and four subsequently at each time point, on postoperative days 3, 7, 21, and 60. None of the animals showed any behavioral changes or neurological deficits suggestive of toxicity and all survived to the scheduled date of sacrifice.

In the rabbit cornea bioassay, none of the corneas had an inflammatory response in the form of corneal edema or neovascularization and remained clear throughout the three weeks of observation.

Gross and microscopic examination of the brain tissue in contact with the implants revealed a tissue reaction to PCPP-SA comparable to that seen in the presence of Gelfoam.

Grossly, there was a fibrous reaction at the craniotomy site which was more pronounced in the animals sacrificed at a later date. The reaction was comparable in the presence of PCPP-SA and Gelfoam. There were no hematomas, cysts, or abscesses.

Microscopically, there was evidence of mild edema, minimal necrosis, and scant polymorphonuclear leukocytes initially, and increasing gliotic changes with time (Table 1). Again, the reaction in the presence of PCPP-SA was comparable to that in the presence of Gelfoam. The differences noted were that by day 7 there was relatively more necrotic changes around PCPP-SA than around Gelfoam and that by day 60 there was better demarcation of the gliotic changes around Gelfoam than around PCPP-SA. The following is a detailed comparison of the histological changes seen at the five time points.

TABLE 1.

Histological Comparison of Polyanhydride Polymer vs Gelfoam Implanted into the Rabbit Brain

Days Post Implantation	PCPP-SA	Gelfoam
1	Mild edema Minimal necrosis Scant polys Fresh hemorrhage	Mild edema Minimal necrosis Scant polys Fresh hemorrhage
3	Mild gliosis Lipid laden macrophages	Mild gliosis Lipid laden macrophages
7	Mild necrosis	Minimal necrosis
21	Decreased gliosis Hemosiderin deposition Lymphs, foamy macrophages and giant cells	Decreased gliosis Hemosiderin deposition Lymphs, foamy macrophages and giant cells
60	Gliotic changes; Somewhat diffuse histiocytes	Gliotic changes; Clearly demarcated gliotic margin histiocytes

Day One

The tissues in contact with either PCPP-SA or Gelfoam showed mild edema, minimal necrosis, fresh hemorrhages, and a few polymorphonuclear leukocytes. These changes were restricted to the site of implantation (Figures 1A and 1B). The polymer was characterized by a well-defined crystalline structural pattern (Figure 1C).

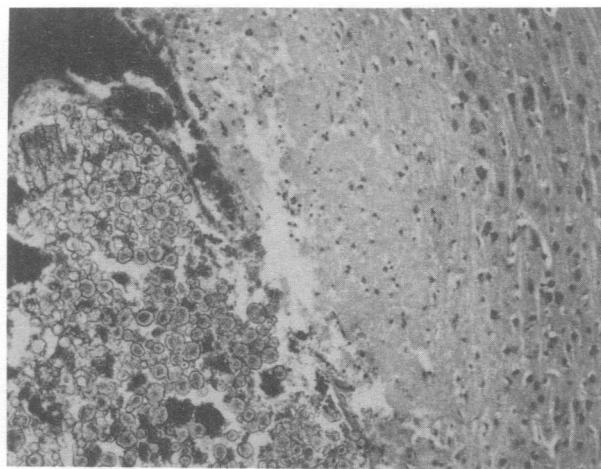


Figure 1A. PCPP-SA (50:50), day 1 (115X)

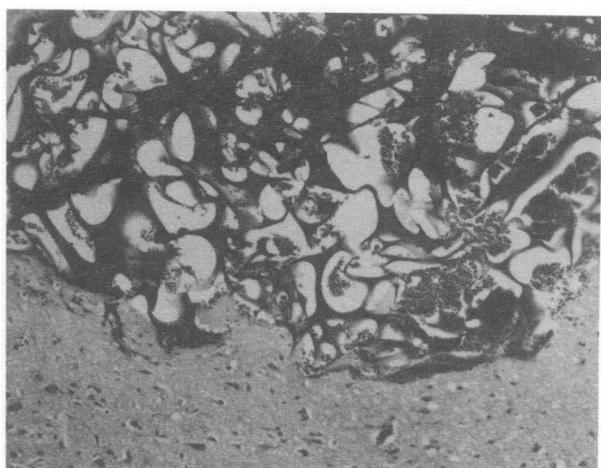


Figure 1B. Gelfoam, day 1 (110X)

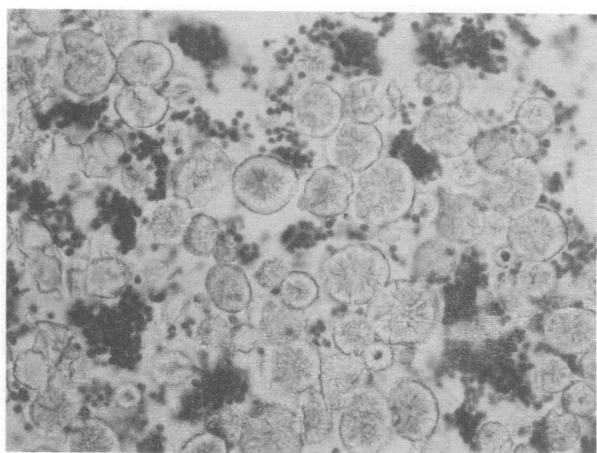


Figure 1C. PCPP-SA (50:50), day 1 (400X)

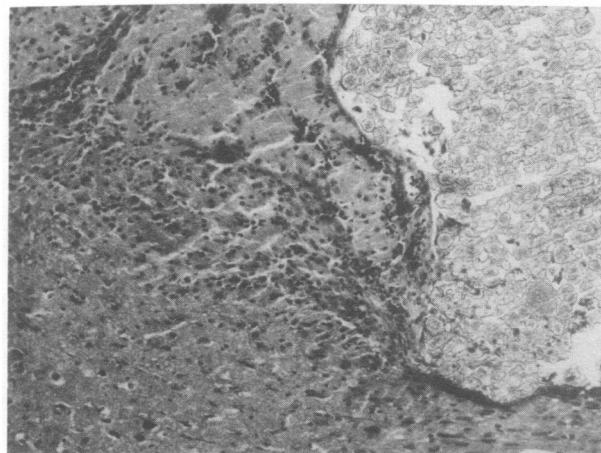


Figure 1D. PCPP-SA (50:50), day 7 (115X)



Figure 1E. Gelfoam, day 7 (115X)

Day Three

Both groups began to develop mild gliosis in the vicinity of the implants. There was still mild edema, minimal necrosis, and scant lipid-laden macrophages in the tissues in contact with the implants. No ischemic neurons were appreciated.

Day Seven

Although both groups showed instances of prominent gliosis, necrosis, and lipid-laden macrophages restricted to the tissues in contact with the implants (Figures 1D and 1E), there was relatively more necrosis in the tissues in contact with PCPP-SA. Ischemic neurons with dark, pyknotic nuclei were appreciated in the necrotic areas. A few giant cells began to appear within the polymer and Gelfoam. The polymer began to lose its crystalline radial pattern.

Day Twenty-One

Both implant groups showed less gliosis and increased hemosiderin deposition (Figures 1F and 1G). Polymorphonuclear leukocytes were substituted by lymphocytes and foamy macrophages, and an increasing number of multinucleated giant cells attempting to engulf fragments of PCPP-SA and Gelfoam were appreciated. The reactive area is extremely well-demarcated from the surrounding normal neural tissue (Figure 1H and 1I). No differences at this stage were seen between the tissues in contact with PCPP-SA and those in contact with Gelfoam.

Day Sixty

By this stage, the biodegradable polymer lost completely its refractile pattern (Figure 1J). Only rare remnants of Gelfoam were appreciated. The gliotic reaction of the tissues in contact with PCPP-SA was less demarcated than the reaction of the tissues

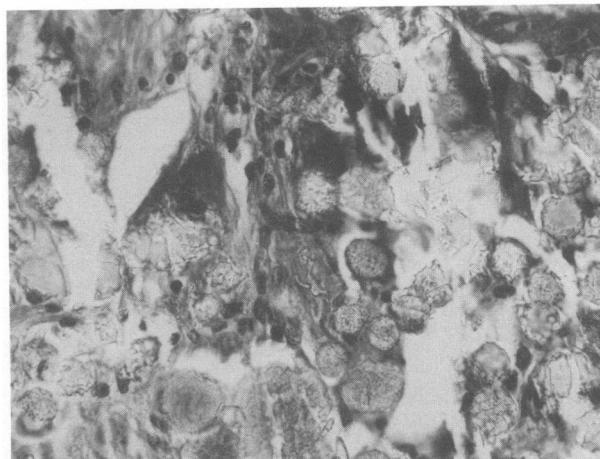


Figure 1F. PCPP-SA (50:50), day 21 (410X)

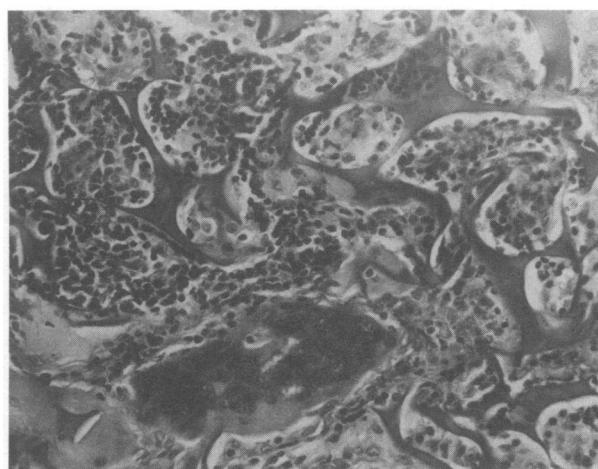


Figure 1G. Gelfoam, day 21 (265X)

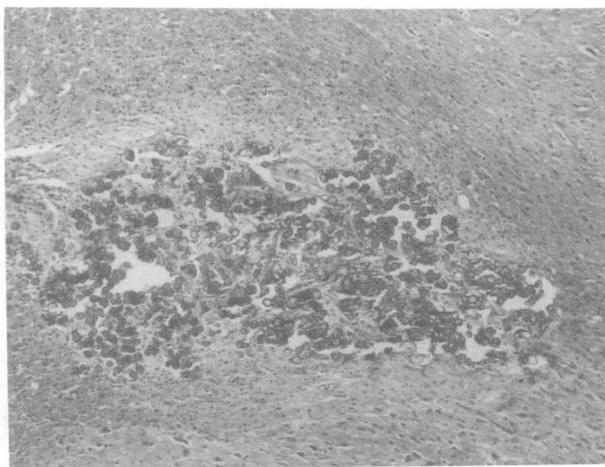


Figure 1H. PCPP-SA (50:50), day 21 (50X)

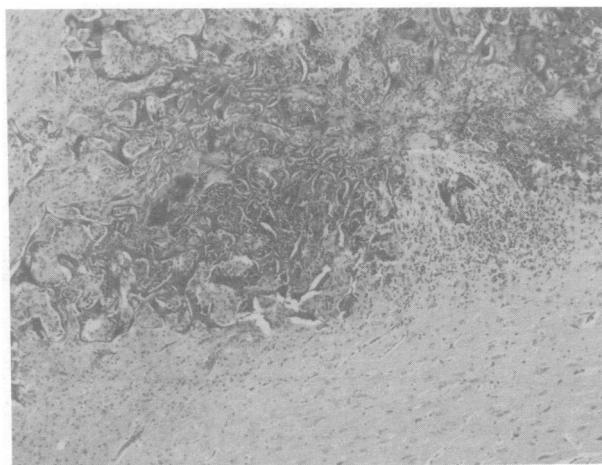


Figure 1I. Gelfoam, day 21 (50X)

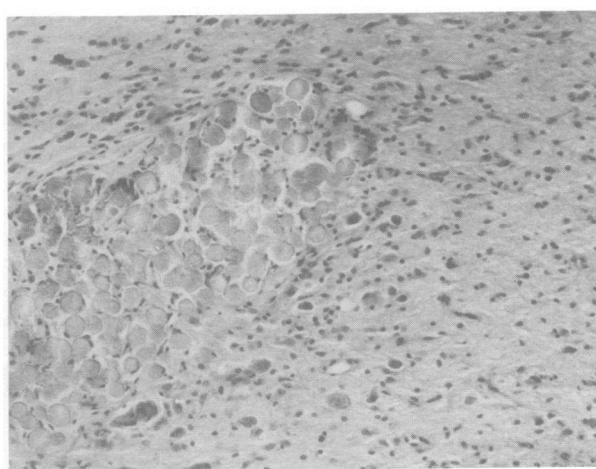


Figure 1J. PCPP-SA (50:50), day 60 (150X)

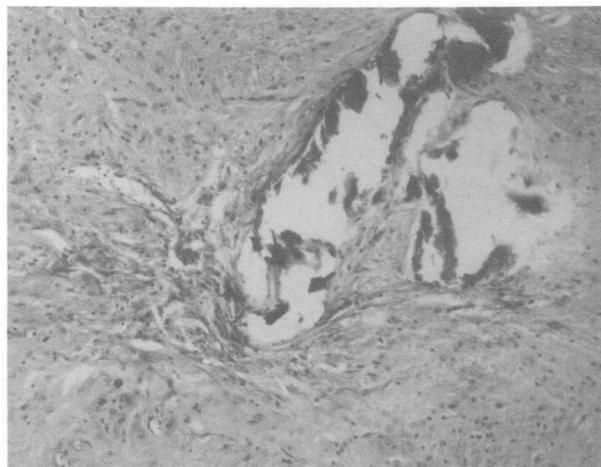


Figure 1K. Gelfoam, day 60 (150X)

with Gelfoam. Multinucleated histiocytes and hemosiderin were still evident at the sites of both PCPP-SA and Gelfoam implantation. One hemisphere showed metaplastic bone formation surrounded by an area of gliosis (Figure 1K).

DISCUSSION

We have shown that a new formulation of a controlled-release carrier, the biodegradable polyanhydride, is biocompatible with the rabbit brain. The sustained, localized administration of drugs incorporated into polymeric devices offers an alternative to systemic administration (8,11). The biodegradable polymers have excellent release kinetics governed by the surface erosion of the matrix and, most importantly, eventually disappear from the site of implantation (7). The recently described biodegradable polyanhydrides (12,13,14) have been shown to be biocompatible both *in vivo*, by implantation in the rabbit cornea and in the rat subcutaneous tissue, and *in vitro*, using bovine aortic endothelial cells and smooth muscle cells (13).

We investigated the biocompatibility of the PCPP-SA polymer with neural tissue because of the potential applications of the biodegradable polyanhydride polymers to neurological surgery. We compared the tissue reaction in the presence of PCPP-SA with that in the presence of absorbable gelatin sponge (Gelfoam) in the rabbit brain. Gelfoam, an absorbable matrix derived from a gelatin preparation, has been shown to induce a tissue reaction comparable to that of a resorbing clot and has been used extensively in neurological surgery with minimal adverse effects (1). It has been used as the standard for evaluating new hemostatic agents. For instance, Rybock and Long (15) established the brain biocompatibility of microfibrillar collagen (Avitene) in the cortex of dogs by comparing it to Gelfoam.

In our study the tissue reaction in the presence of the biodegradable polyanhydride PCPP-SA (50:50) was not significantly different from the tissue reaction in the presence of Gelfoam. There were no adverse behavioral or neurological effects noted in any of the animals. The only histological differences between PCPP-SA and Gelfoam were slightly more necrosis around the

polymer on day 7 and a better-demarcated gliotic reaction around Gelfoam in general. Subsequent to day 7, however, there was no difference in the extent of necrosis seen with the two implants. The initial increase of necrosis around the polymer may be due to a faster degradation of the polymeric matrix with accumulation of acidic breakdown products at the site. The degradation rate of this polymer is dependent on the ratio between PCPP and SA. PCPP degrades very slowly, taking months to completely disappear. SA, on the other hand, degrades rapidly, disappearing in days to weeks. PCPP-SA (50:50) takes about 4-5 months to degrade completely in vitro (12). Different formulations of the polyanhydride with a higher percentage of SA may elicit a different histological reaction, but we conclude that the 50:50 formulation tested in this study is biocompatible.

In summary, we report the biocompatibility of a biodegradable polyanhydride, PCPP-SA (50:50), with the rabbit brain. The tissue reaction to the polymer was not significantly different from that observed in the presence of Gelfoam, a standard hemostatic agent used routinely in neurological surgery. These polymeric devices for the localized, sustained release of drugs may have a major impact on the pharmacological applications used as an adjunct to neurological surgery. For example, the polymers could be used for the interstitial delivery in the brain of chemotherapeutic agents such as 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), anti-edema agents such as dexamethasone, and biological response modifiers such as angiogenesis inhibitors (2,3,5,9). The more prolonged degradation (i.e., 4-5 months) of the 50:50 PCPP:SA may prove optimal for an intracranial release of some of these agents.

ACKNOWLEDGMENTS

Supported in part by NIH Grant No. NS01058-01, American Cancer Society Grant No. IN-11W, the Andrew W. Mellon Foundation Johns Hopkins University Faculty Development Award, the Association for Brain Tumor Research Fellowship in Memory of Steven Lowe, and NIH Grant No. GM26698. We thank Michael Pinn and Carla Reinhard for technical assistance.

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