Brain biocompatibility of a biodegradable, controlled-release polymer in rats

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We report the biocompatibility in the rat brain of a controlled-release, biodegradable polymer, the polyanhydride poly-[bis(*p*-carboxyphenoxy)propane-sebacic acid] copolymer (PCPP-SA) in a 20:80 formulation. The biodegradable polyanhydride can be used for drug delivery directly into the brain, circumventing the difficulties posed by the blood–brain barrier and avoiding the consequences of having to administer toxic doses systemically to reach therapeutic doses in the central nervous system. The tissue reaction in the presence of PCPP-SA was compared to that seen with other standard neurosurgical implants. Fifty-six adult Sprague-Dawley rats were assigned to one of seven groups and underwent bilateral frontal lobe implantation of PCPP-SA (42 hemispheres), Surgicel (oxidized regenerated cellulose) (35 hemispheres), or Gelfoam (absorbable gelatin sponge) (35 hemispheres). None of the animals showed any behavioral changes or neurological deficits suggestive of either systemic or localized toxicity from the biodegradable polyanhydride, all surviving to the scheduled data of sacrifice. PCPP-SA evoked a well localized inflammatory reaction, comparable to that of Surgicel, which resolved as the PCPP-SA polymer degraded over five weeks. The biodegradable polyanhydride has been shown in this study to be nontoxic and biocompatible in the rat brain, when compared to standard neurosurgical implants.

INTRODUCTION

We have developed a system for interstitial drug delivery in the brain that circumvents the difficulties posed by the blood–brain barrier (BBB) and avoids the consequences of having to administer toxic doses of drugs systemically to reach therapeutic doses in the central nervous system (CNS). This system consists of incorporating drugs into solid biodegradable polymers, which can then be surgically implanted in the brain to release the drug locally, in a sustained and controlled fashion, for periods ranging from days to years.

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Polymeric devices for the sustained administration of biologically active agents have been proposed as an alternative to current methods of drug delivery.[1–3] Of the numerous formulations available, the synthetic biodegradable polymers, as first described by Yolles et al.,[4,5] are devices in which the drug is liberated as the polymeric matrix is gradually degraded.[5] These devices have the advantage of obviating the complications of a permanent foreign body implant. Recently, a new class of these polymers, the biodegradable polyanhydrides, have been developed.[6,7] The biodegradable polyanhydrides have excellent release kinetics and degradation products that are nonmutagenic, noncytotoxic, and nonteratogenic.[8] 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* by use of bovine aortic endothelial cells and smooth muscle cells.[8]

A biodegradable polymeric delivery system may have numerous applications in neurological surgery, where (i) pathology in the brain or spinal cord is frequently localized, (ii) the presence of the BBB limits pharmacological access to the central nervous system, and (iii) the systemic administration of drugs destined to reach the CNS carries significant and unwanted generalized side effects. We therefore evaluated the biocompatibility in the rat brain of a biodegradable polyanhydride, poly[bis(*p*-carboxyphenoxy)-propane-sebacic acid] copolymer (PCPP-SA) in a 20:80 formulation. We compared the tissue reaction in the presence of PCPP-SA to that seen with absorbable gelatin sponge (Gelfoam) or with oxidized regenerated cellulose (Surgicel), two standard agents implanted routinely in neurological surgery for purposes of hemostasis.[9] Since Gelfoam lacks any intrinsic hemostatic properties, it is usually soaked in thrombin and then applied to the bleeding site, thus serving as a drug-delivery matrix.[9] The release of thrombin from Gelfoam, however, is immediate and poorly controlled.

MATERIALS AND METHODS

Animals

Fifty-six adult male Sprague-Dawley rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and kept in standard animal facilities, four rats per cage, with free access to Certified Rodent Chow No. 5002 (Ralston Purina, Co., St. Louis, MO) and Baltimore City water. The rats were randomly assigned to one of seven groups, each group containing eight rats, to be sacrificed at different dates from the time of implantation.

Implants

PCPP-SA (20:80) disks were obtained from Nova Pharmaceutical Corporation, Baltimore, Maryland. The polymer was synthesized by melt polycondensation and formulated by compression molding into disks weighing

about 12 mg, measuring 3 mm in diameter and 1 mm in height.[7] Under the surgical microscope, the disks were cut into cubes measuring about $1.5 \times 1.5 \times 1.0$ mm^3, which were subsequently sterilized under UV light for about 2 h. The pieces were kept at −30°C until the day of implantation.

Gelfoam (The Upjohn Company, Kalamazoo, MI) and Surgicel (Johnson and Johnson, New Brunswick, NJ) were cut into pieces of similar volume and were subsequently sterilized under UV light for about 2 h.

Anesthesia

The animals were anesthetized intraperitoneally with 3–5 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.

Inplantation technique

The head was shaved and prepared with 70% ethyl alcohol and Prepodyne solution. Using microsurgical technique, a midline incision was made, the periosteum removed, and the sagittal and coronal sutures identified. Bilateral burr holes 3 mm in diameter were made at the coronal suture 2–3 mm off the midline without damaging the underlying dura. An avascular region of the cortex was selected, and an incision about 3 mm deep was made with a No. 11 blade. The bleeding was allowed to subside spontaneously and the excess blood was removed by microsuction. Extreme care was taken not to touch the cortex with any materials other than the blade, the jeweler's forceps, and the implants. Once the bleeding subsided, the implants were introduced into the cortical defect. In each of the 7 groups, 6 pieces of PCPP-SA polymer, 5 pieces of Surgicel, and 5 pieces of Gelfoam were implanted following the assignments listed in Table I.

After the introduction of the implant, the dural edges were reapproximated, and the skin was closed with surgical staples. The animals were returned to their cages when fully awake.

TABLE I

Rat Number	Right Hemisphere	Left Hemisphere
1	PCPP-SA	Gelfoam
2	Surgicel	PCPP-SA
3	PCPP-SA	Surgicel
4	Gelfoam	PCPP-SA
5	PCPP-SA	Gelfoam
6	Surgicel	PCPP-SA
7	Gelfoam	Surgicel
8	Surgicel	Gelfoam

Daily evaluation

The 56 rats were examined twice daily after surgery. Particular attention was given to (i) behavioral changes manifested by decreased alertness, passivity, impaired grooming, restlessness, irritability, or fearfulness, and to (ii) neurological deficits manifested by focal motor changes or gait disturbances.[10]

Histology

The animals were sacrificed by an ether overdose. The seven groups were sacrificed sequentially on postoperative days 3, 6, 10, 15, 21, 28, and 36.

The brains were removed, fixed in 10% phosphate buffered formalin for 10–14 days, cut coronally, embedded in paraffin, sectioned with a microtome, and stained with hematoxylin and eosin. A qualitative assessment of the inflammatory reaction in the region of the implants was carried out. The acute or chronic elements of the inflammatory response were described. Other generalized features of the inflammatory response, such as its extent (localized versus generalized), gliosis, edema, coagulative necrosis, and hemorrhage, were noted. The inflammatory features were qualified in three categories as being "severe," "moderate," or "mild or minimal." The histological appearance of the degrading implants were noted.

RESULTS

None of the animals showed any behavioral changes or neurological deficits suggestive of either systemic or localized toxicity from the biodegradable polyanhydride. The variable combinations of the implants did not affect the response elicited by the individual implants. All rats survived to the scheduled date of sacrifice.

The course of the inflammatory changes around the implants was divided into three stages: acute, subacute, and chronic. Briefly, during the acute phase—characterized by the predominance of polymorphonuclear leukocytes—the PCPP-SA polymer induced the most pronounced reaction, followed by Surgicel, and then by Gelfoam. In the subacute phase—characterized by the predominance of histiocytes—Surgicel was associated with the most pronounced response, followed by Gelfoam, and then the PCPP-SA polymer. In the chronic phase—characterized by maximal degradation of the implants—Surgicel caused the most pronounced reaction, followed by the PCPP-SA polymer, and then Gelfoam. The following three sections describe, in more detail, the histological changes associated with each of the implants as they progressed from the acute inflammatory phase to the subacute and then chronic phases.

Acute phase: Days 3–6

The PCPP-SA polymer, which decomposes rapidly in the presence of water, evoked a moderate to severe acute response that peaked around day 3 (Fig. 2a). By day 3, the tissues surrounding the PCPP-SA polymer showed an acute, localized inflammatory reaction with moderate edema and, in a few specimens, minimal coagulative necrosis. The PCPP-SA polymer itself appeared as a cribriform matrix. This lattice was composed of eosinophilic, erythrocyte-like, spherical units. A yellowish, highly refractile, crystalline material was seen in the interstices (Fig. 1).

Surgicel evoked a moderate to severe acute response, which peaked around days 3–6 and was most pronounced within the implant (Fig. 3a). This response, already evident by day 3, was accompanied by mild to moderate edema, mild gliosis, and minimal coagulative necrosis in a few specimens. By day 6, a moderate to severe acute inflammatory reaction was still in progress within the Surgicel matrix. A severe but localized histiocytic reaction was noted around the implant, heralding the transition into the subacute inflammatory phase. This reaction was associated with moderate edema. The Surgicel matrix, however, remained essentially intact.

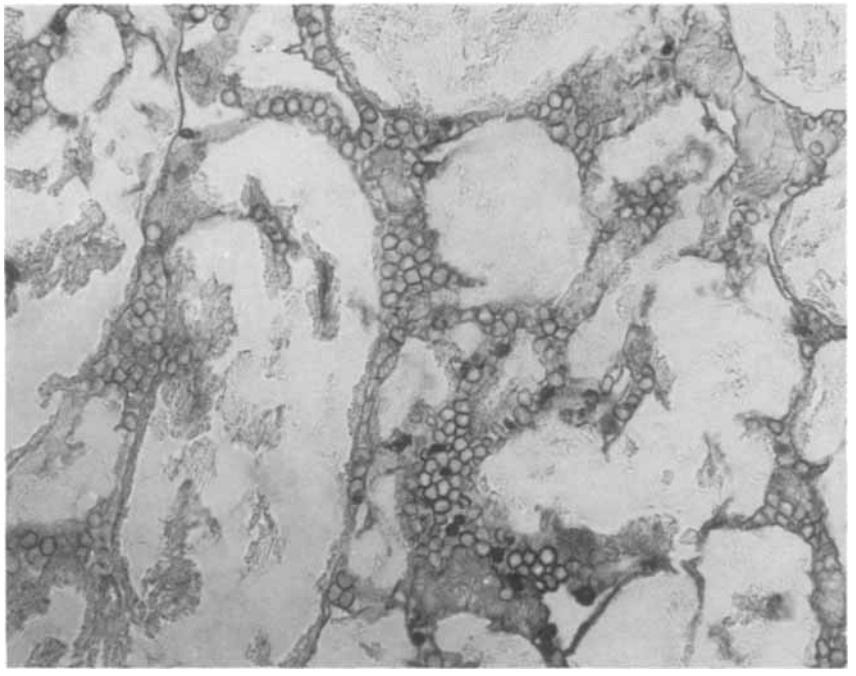

Figure 1. Hematoxylin and eosin stain of the PCPP-SA polymer consisting of a cribriform matrix containing eosinophilic spherical units and a refractile yellow crystalline material within the interstitial spaces (original magnification ×425).

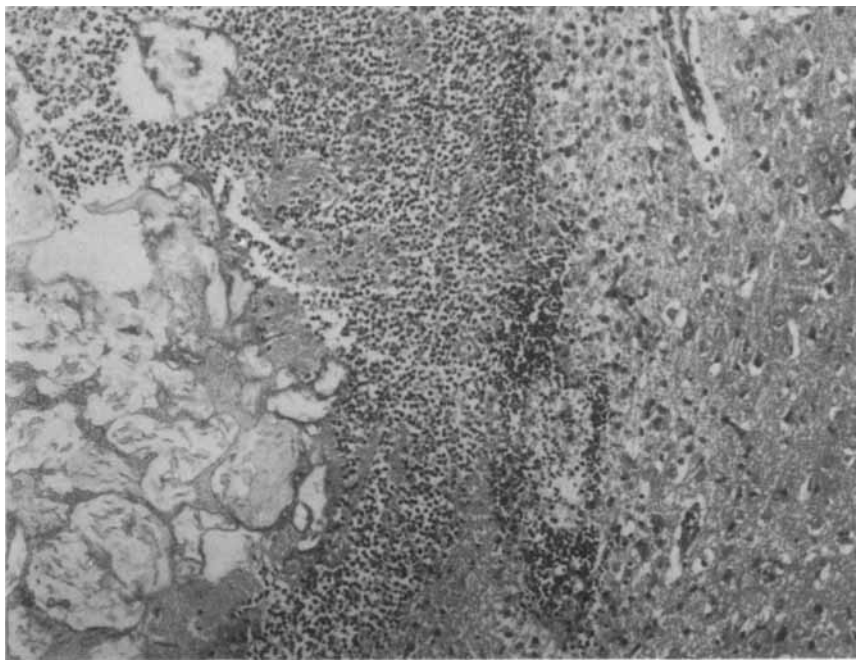

Figure 2a. Day 3. PCPP-SA polymer (left) surrounded by a localized acute inflammatory response. Adjacent brain shows moderate edema (original magnification ×125).

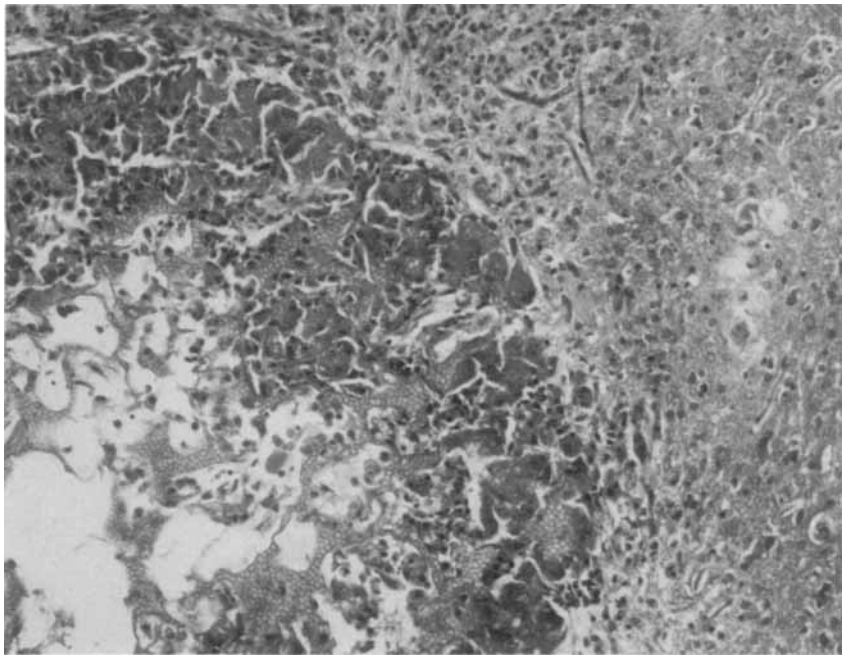

Figure 2b. Day 15. PCPP-SA polymer (left) infiltrated by numerous epithelioid histiocytes, with surrounding brain showing lymphocytes, edema, and pale foamy histiocytes (original magnification ×135).

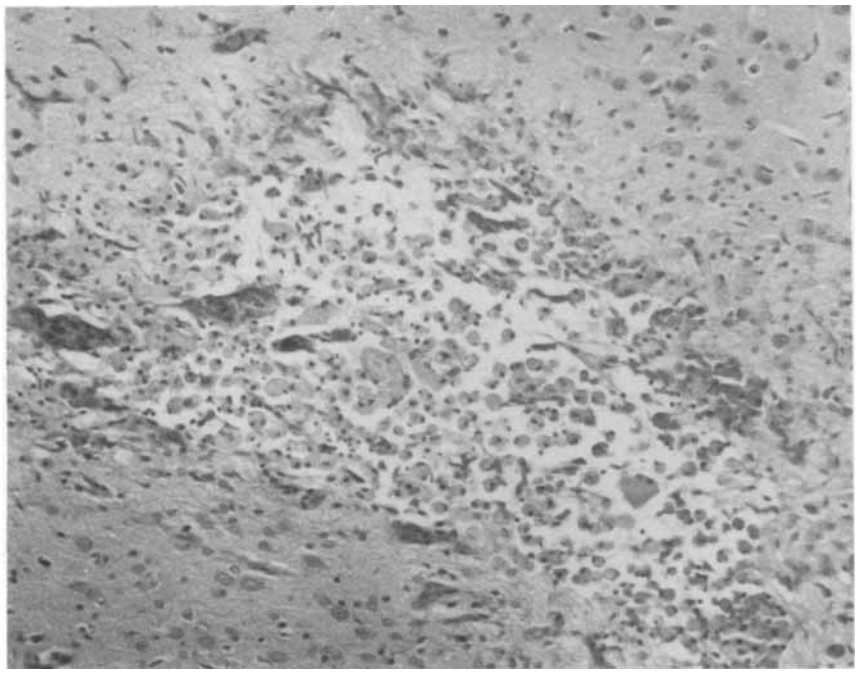

Figure 2c. Day 36. PCPP-SA polymer implantation site shows only discrete collection of hemosiderin-laden, pale foamy macrophages (original magnification ×160).

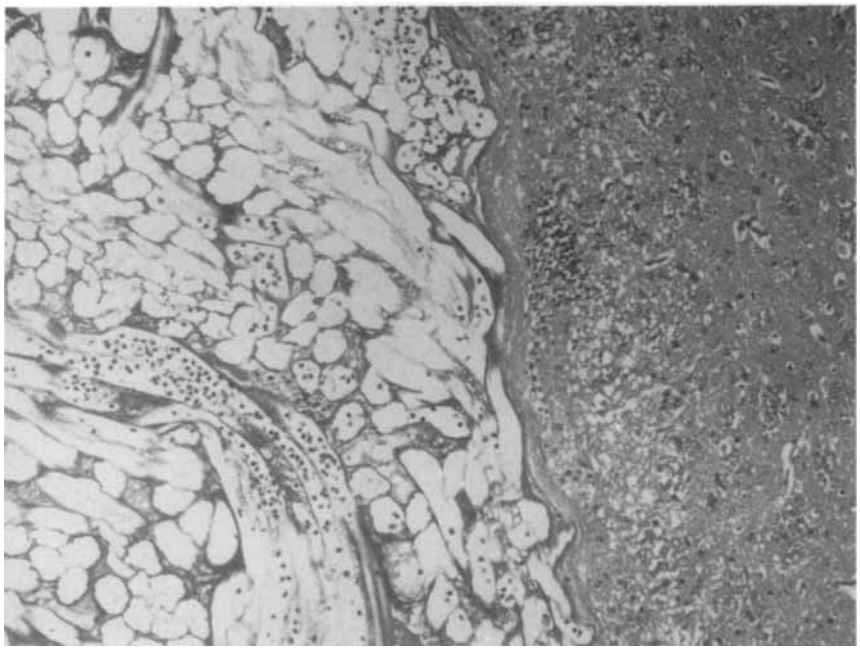

Figure 3a. Day 3. Surgicel (left) infiltrated by neutrophils with surrounding brain showing edema (original magnification ×135).

Figure 3b. Day 15. Surgicel (left) surrounded by numerous pale foamy and hemosiderin-laden macrophages as well as lymphocytes (original magnification ×135).

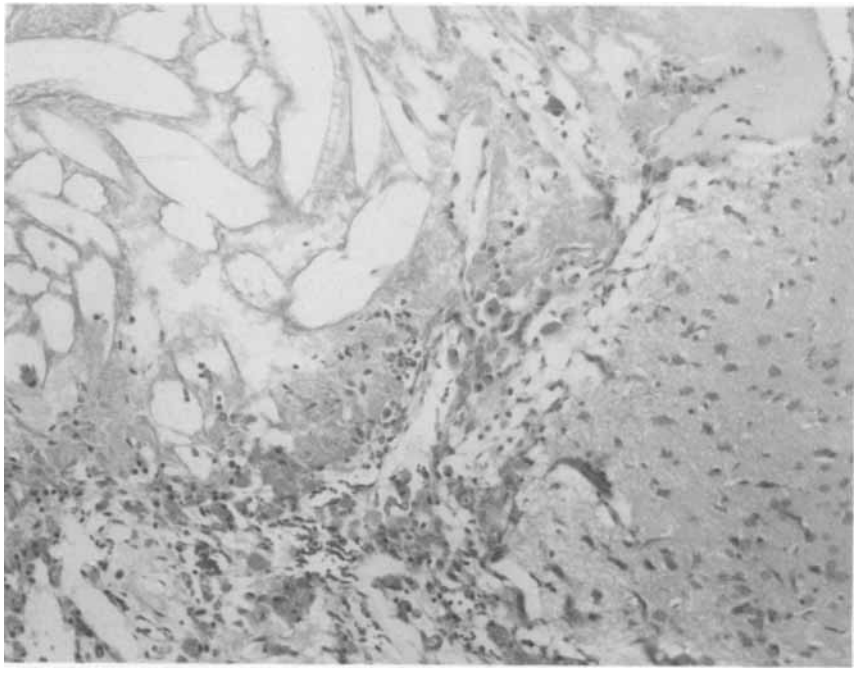

Figure 3c. Day 36. Persistent degrading Surgicel (left) surrounded by hemosiderin-laden macrophages and scattered lymphocytes (original magnification ×160).

In contrast to the PCPP-SA polymer and Surgicel, Gelfoam provoked a mild acute inflammatory response, which peaked around days 3–6 (Fig. 4a). By day 3, the tissues in contact with Gelfoam had only minimal inflammatory change with minimal to moderate edema, recent hemorrhage, and minimal coagulative necrosis in a few specimens. By day 6, the region surrounding Gelfoam still had minimal acute inflammatory changes and only minimal edema. A moderate to severe, well-demarcated histiocytic reaction, however, was noted around the implant by day 6.

Subacute phase: Days 6–10

The subacute inflammatory phase of the PCPP-SA polymer was less pronounced than that of both Surgicel and Gelfoam (Fig. 2b). By day 6, the acute inflammatory reaction around the PCPP-SA polymer subsided, leaving behind fragmented neutrophils and their nuclear debris. Histiocytes became more numerous, creating a well-demarcated region surrounding he PCPP-SA polymer. There was a minimal rim of edema around the histiocytic reaction. Some specimens had regions characterized by a moderate foamy histiocytic reaction and moderate coagulation necrosis. The PCPP-SA polymer's cribriform matrix retained the features seen at 3 days but looked paler, suggesting early degradation of the matrix. The crystalline material within the lattice degraded to a greater degree. By day 10, the

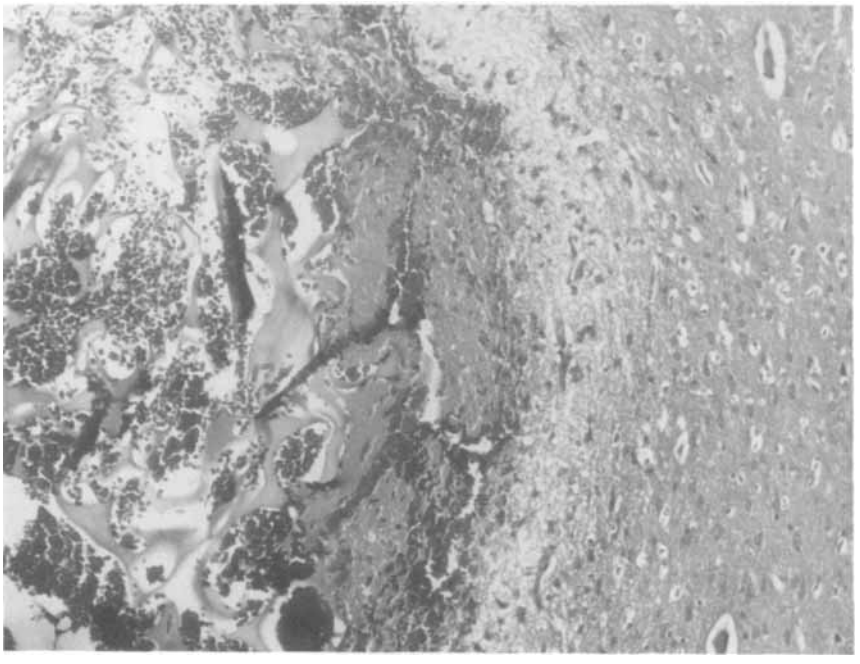

Figure 4a. Day 3. Gelfoam (left) with minimal adjacent necrosis and moderate edema (original magnification ×135).

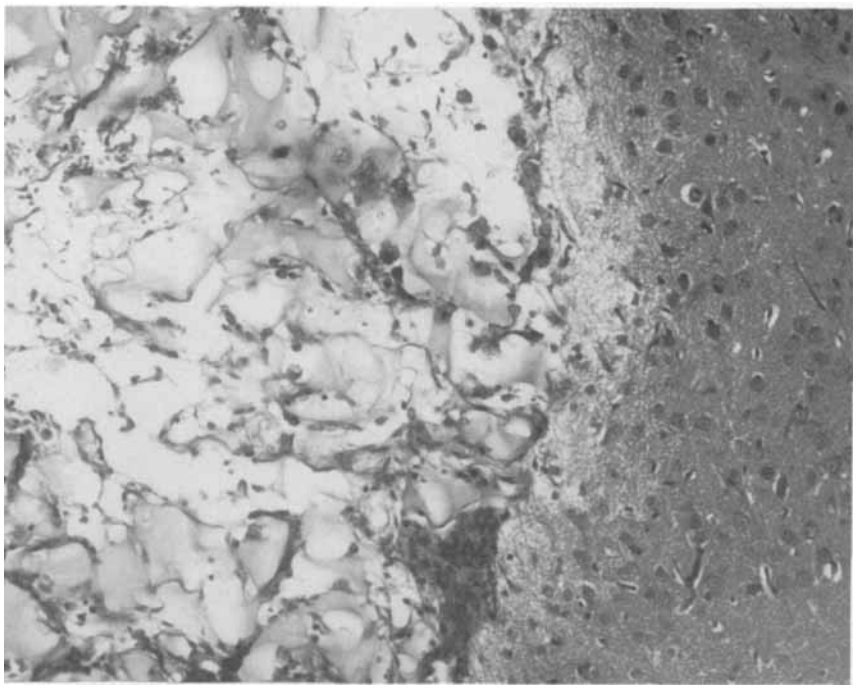

Figure 4b. Day 15. Gelfoam (left) surrounded by a focal collection of histiocytes and minimal edema (original magnification ×180).

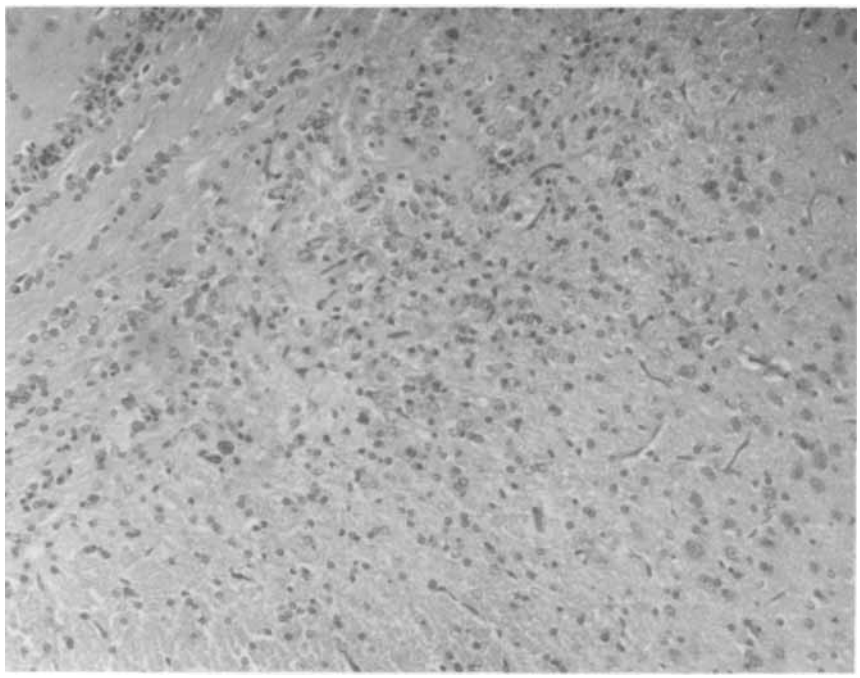

Figure 4c. Day 36. Gelfoam implantation site showing only mild increased cellularity due to scattered hemosiderin-laden macrophages. (original magnification ×160).

well-demarcated histiocytic reaction around the PCPP-SA polymer persisted. There was minimal edema outside the histiocytic infiltrate. As before, the cribiform matrix of the PCPP-SA polymer appeared degraded, with barely visible, yellow, refractile components seen in the interstices. In two specimens, the PCPP-SA polymer and its surrounding inflammatory infiltrate appeared similar to those specimens removed on days 3 and 6 with more intact PCPP-SA polymer and a more pronounced acute inflammatory response.

The subacute reaction of Surgicel, which peaked between days 6 and 10, was moderate to severe, similar to the reaction associated with the PCPP-SA polymer. By day 10, the Surgicel implants were surrounded by a moderate, well-demarcated histiocytic response. Many of the histiocytes contained hemosiderin, and a moderate amount of edema. Some specimens had a minimal amount of coagulative necrosis adjacent to the implant. The Surgicel itself showed slight evidence of degradation.

Gelfoam was associated with a mild subacute response, which peaked around days 6–10 (Fig. 4b). By day 15, the response to Gelfoam was still mild, characterized by focal areas of histiocytes and rare multinucleated giant cells engulfing the material. A minimal amount of edema and focal hemosiderin deposition was noted. By day 21, Gelfoam was barely identifiable and even absent in some sections. A minimal number of hemosiderin-laden histiocytes and a moderate amount of edema were noted. No lymphocytic infiltrate was noted.

Chronic phase: Days 15–36

The PCPP-SA polymer degraded totally by day 36, at which time only a small focus of hemosiderin was evident. Two of the six implants lagged behind the others, but also decomposed totally by day 36 (Fig. 2c). By day 15, the tissues in contact with the PCPP-SA polymer still showed a well-demarcated, chronic inflammatory response, consisting of eosinophilic epithelioid histiocytes, pale foamy histiocytes, and lymphocytes. Several multinucleated histiocytes could be identified trying to engulf the degrading polymeric matrix. There was a mild amount of edema around the PCPP-SA polymer. The cribriform matrix of the PCPP-SA polymer appeared further degraded, and the interstitial refractile component was not visible. By day 21, there was still a well-demarcated, moderate histiocytic infiltrate around the PCPP-SA polymer. Most of the histiocytes were mononuclear with some multinucleated forms. The histiocytes varied in appearance from plump epithelioid, to pale foamy, to those containing hemosiderin. In addition, a moderate number of lymphocytes were noted. There was a moderate amount of edema. The PCPP-SA polymer appeared totally degraded within the center but retained a semblance of its cribriform matrix toward the periphery. By day 28, minute amounts of residual PCPP-SA polymer were seen within histiocytes in several cases. A well-demarcated area of scattered, hemosiderin-laden histiocytes and minimal edema remained at

the implantation site. By day 36, the PCPP-SA polymer implant sites had discrete foci of hemosiderin-laden macrophages and small collections of lymphocytes. There was only mild edema. One specimen had evidence of early microcystic changes and neovascularization. No residual polymer was seen.

By contrast to the PCPP-SA polymer and Gelfoam, Surgicel was still present on day 36, surrounded by hemosiderin, a few lymphocytes, and a few multinucleated giant cells (Fig. 3c). By day 15, Surgicel was surrounded by a well-demarcated, severe, mixed histiocytic and lymphocytic response (Fig. 3b). Numerous pale, foamy, and hemosiderin-laden histiocytes were present. A moderate amount of edema was noted. By day 28, the Surgicel matrix remained recognizable, although degraded. There was a focal, mild to moderate histiocytic reaction with scattered lymphocytes and minimal edema. By day 36, degrading Surgicel was still present. It was surrounded by a focal collection of hemosiderin-laden histiocytes and scattered lymphocytes associated with mild edema. Scattered multinucleated giant cells could be seen engulfing the material. Regions with microcystic changes were present.

The chronic reaction associated with Gelfoam was characterized by the presence of slightly more multinucleated giant cells than seen surrounding the PCPP-SA polymer and Surgicel. By day 21 some Gelfoam implants had degraded totally, and by day 28, most implants were resorbed. By day 36, all implants were resorbed (Fig. 4c). By day 28, little residual Gelfoam was seen. Minimal edema and minimal hemosiderin deposition were noted at the implant site. By day 36, the implantation site was well healed and inconspicuous. The only remnants of an inflammatory response were occasional hemosiderin-laden macrophages and a suggestion of focal edema.

DISCUSSION

We have shown that the biodegradable polyanhydride PCPP-SA (20:80) implanted interstitially in the brain of rats is biocompatible and degrades within 36 days of implantation, without causing any systemic or neurologic toxicity. The localized inflammatory reaction evoked by the PCPP-SA polymer was comparable to that seen in the presence of Surgical, but more marked than that seen in the presence of Gelfoam. This study suggests that this polymer could be implanted safely in the brain for the interstitial delivery of drugs within the CNS.

Drug delivery to the brain and spinal cord presents unique problems. The CNS bioavailability of systemically administered drugs is determined by the BBB.[11] This feature has limited the drugs that can be used against CNS pathology to those that are highly lipid soluble, have a low ionization potential in physiologic buffers, and exhibit minimal binding to plasma proteins.[12] The limited number of antibiotics that can be used against brain abscesses and of chemotherapeutic agents that are effective against brain tumors is a result of the pharmacological constraints posed by the BBB. Moreover,

given that CNS pathology is often localized,[13] it is unfortunate to have to administer toxic doses systemically to reach therapeutic doses in the CNS.

The biodegradable polyanhydride tested in this study, PCPP-SA, is made up of two monomeric units: *p*-carboxyphenoxy propane (PCPP), which is relatively hydrophobic, and sebacic acid (SA), which is more hydrophilic. A polyanhydride consisting only of PCPP degrades very slowly and may take years to dissolve completely. Polymerization of SA, by contrast, results in a matrix that dissolves rapidly over a few days. Copolymerization of PCPP with SA yields a biodegradable polyanhydride whose degradation rate can be altered by shifting the ratio of the two monomers.[7] The formulation used in this study contained 20% PCPP and 80% SA (w/w), which degraded completely in 5 weeks. Other formulations, containing a higher percentage of PCPP, will take longer to degrade. Thus, the synthesis of the PCPP-SA polymer can be varied to obtain shorter or longer periods of drug delivery, as needed. The hydrophobic, anhydrous environment within the PCPP-SA polymer should protect the drug from premature degradation and eventually will release bioactive drug at the polymer–tissue interface.

Polymeric devices for the sustained administration of biologically active agents are well suited for the interstitial delivery of drugs in the brain. The biodegradable polymers liberate drug as the polymeric matrix is gradually eroded.[5] The biodegradable polyanhydrides have excellent release kinetics—presumably due to pure surface erosion as opposed to bulk erosion in other biodegradable polymers—and have been shown to be biocompatible in standard *in vivo* and *in vitro* assays.[6–8] Clinical trials are currently underway using this PCPP-SA polymer to deliver chemotherapeutic agents intracranially to treat malignant brain tumors.

In conclusion, the biodegradable polyanhydride PCPP-SA in a 20:80 formulation is biocompatible with the rat brain. The transient inflammatory reaction elicited by the PCPP-SA polymer as it degrades is comparable to that seen in the presence of Surgicel but slightly more pronounced than that seen in the presence of Gelfoam. Like Surgicel, a well-established hemostatic agent used routinely in neurosurgery, the PCPP-SA polymer elicits a transient, well-demarcated tissue reaction which subsides as the foreign substance is degraded. None of the rats tested showed any evidence of systemic or neurologic toxicity and all lived to the scheduled date of sacrifice. The PCPP-SA polymer degraded totally by 36 days. An implantable, biodegradable polymeric delivery system may have varied applications in neurosurgery.

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