Journal of Controlled Release, 19 (1992) 325–330

COREL 00692

Brain biocompatibility of a biodegradable controlled release polymer consisting of anhydride copolymer of fatty acid dimer and sebacic acid

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(Received 28 June 1991; accepted in revised form 23 September 1991)

The development of new biodegradable polymers (e.g. PCPP-SA) has allowed the prolonged controlled release of lipid soluble chemotherapeutic agents in the laboratory and clinically. In order to optimize this approach, a new polymer has been developed (the copolymer of fatty acid dimer and sebacic acid p[FAD-SA]) to release water soluble agents. In this study we sought to determine the safety and biocompatibility of this new polymer in the brain. We compared the tissue reaction to this polymer to the reaction observed with oxidized cellulose (Surgicel^R) and with absorbable gelatin sponge (Gelfoam^R).

Fifty-six adult Fischer 344 rats were assigned to 1 of 7 groups and underwent bilateral 3 mm burr holes. Neurological or behavioral changes were assessed daily and the groups were killed sequentially on postoperative days 3, 6, 10, 15, 21, 28, and 36. No neurological deficits or behavioral changes suggestive of either systemic or localized toxicity were observed in the animals implanted with the new polyanhydride. A well demarcated acute inflammatory response was seen at day 3 and 6 for p(FAD-SA) and Surgicel^R implants. The inflammatory response remained well localized and resolved with total degradation of the polymer by day 36.

The localized reaction evoked by this polymer was comparable to the one of the previously studied biodegradable polymers (PCPP-SA) and to the response to commonly used surgical hemostatic materials such as Surgicel^R. The biodegradable copolymer of fatty acid dimer and sebacic acid may play an important role in drug delivery to the brain by by-passing the blood-brain barrier to administer water soluble agents directly to the brain.

Key words: Biodegradable polymers; Brain biocompatibility; Anticancer drugs

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Introduction

The brain has a natural "protection" system, the blood-brain barrier, that prevents unwanted

substances or toxins from entering the brain parenchyma. Unfortunately, this barrier also limits the entry of therapeutic agents which would benefit the brain in treating cerebral disorders. We have developed a method for delivering sustained, high doses of drugs to specific areas of the brain [1]. We have previously utilized a biodegradable polymer, poly-bis-(*p*-carboxyphenoxy)-propane sebacic acid [2–5], with chemotherapeutic agents [6], dexamethasone [7,8], heparin, and cortisone [9] to directly deliver high concentrations of drugs to the brain over prolonged periods of time.

The safety of this polyanhydride delivery system has been demonstrated clinically [10] and a clinical placebo-controlled study for delivery of BCNU, a nitrosourea chemotherapeutic drug, is currently underway.

In order to optimize the delivery of water soluble drugs, a new biodegradable anhydride copolymer of fatty acid dimer (FAD) and sebacic acid (SA), poly(FAD:SA) has been developed and shown to be capable of controlled release of water soluble chemotherapeutic drugs such as methotrexate, carboplatin, and 4-hydroperoxycyclophosphamide (4-HC) to the rat brain [11]. In vitro release kinetics studies conducted on water soluble drugs have shown sustained release of the drugs over a period of 3 to 5 weeks, paralleling the degradation time of the polymer.

The safety and the biocompatibility of this biodegradable polymer was studied in the rat brain and compared with that of two commonly used neurosurgical biodegradable hemostatic implants, oxidized cellulose (Surgicel^R) and the absorbable gelatin sponge (Gelfoam^R) [12].

Materials and Methods

Fifty-six adult Fischer 344 rats weighing 250 to 300 g were assigned to 1 of 7 groups, each group containing 8 rats, to be sacrificed at different dates from the time of implantation. The animals were anesthetized with an intraperitoneal injection of 3–5 ml/kg of a solution containing ketamine hydrochloride 25 mg/ml, xylazine 2.5 mg/ml and 14.25 ethyl alcohol in normal saline.

Polymer preparation

A copolymer of fatty acid dimer and sebacic acid was prepared from the prepolymers of FAD and sebacic acid by melt polycondensation. The prepolymers primarily consisted of a mixed anhydride of the diacid with acetic anhydride. The polymer mixture (50:50 FAD:SA) was formulated into rods by melt-casting technique (at 70°C), and subsequently cut under surgical microscope into pieces about $1.5 \times 1.5 \times 1.0$ mm for implantation. Similar size pieces of Surgicel (Johnson and Johnson, New Brunswick, NJ) and Gelfoam^R (The Upjohn Co., Kalamazoo, MI) were also prepared for implantation. All the specimens were sterilized under UV light for 2 h.

Implantation technique

Bilateral burr holes 3 mm in diameter were made at the coronal suture 2–3 mm off the midline. A no. 11 blade was used to make an incision in the cortex about 3 mm in depth. Once the occasional mild bleeding subsided, the implants were introduced into the cortical defects.

Evaluation

The rats were examined twice daily after surgery for neurological deficits or behavioral changes. The 7 groups were sacrificed sequentially on postoperative days 3, 6, 10, 15, 21, 28 and 36; the brains were removed for histological processing. The sections were stained with hematoxylin and eosin and a qualitative assessment of the inflammatory reaction in the region of the implants was carried out. The histological appearance of the degrading implants was also observed.

Results

All the animals survived to the scheduled date of sacrifice with no evidence of behavioral

changes or neurological deficits suggestive of toxicity.

The inflammatory changes around the p(FAD-SA) implants were divided into three stages: acute, subacute, and chronic. In the *acute phase* (days 3–6) the p (FAD-SA) polymer caused a well demarcated inflammatory response characterized by a zone of acellular necrosis surrounding the polymer and focal collections of neutrophils, some of which were undergoing karyorrhexis. There was surrounding edema with scattered xanthomatous macrophages (Fig. 1a). After 6 days the polymers were surrounded by a demarcated area of fibroblastic and histiocytic response with neovascularization and some edema. In the center of the polymer there was necrotic debris with karyorrhexis and degenerating neutrophils. Surgicel^{R} caused a similar demarcated reaction, although less inflammation was seen within the center of the implant (Fig. 2a). The reaction to Gelfoam^{R} was less pronounced (Fig. 3a).

In the *subacute phase* (days 6–15) the inflammatory response characterized by the presence of lymphocytic infiltrates and vacuolar macrophages was again moderate but well demarcated in the case of p(FAD-SA) and Surgicel^{R} and mild in the case of Gelfoam^{R}. Within the center of the p(FAD-SA) polymer, there was karyorrhexis with necrotic debris and no intact neutrophils.

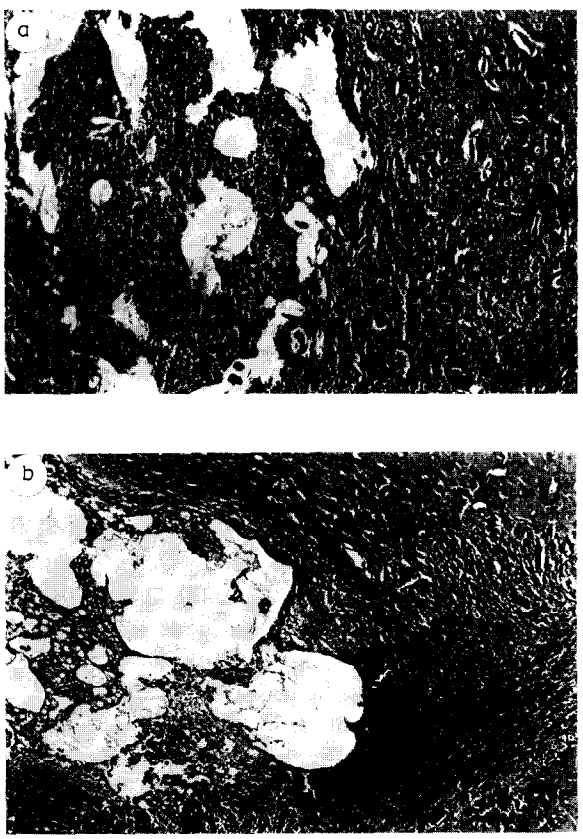

Fig. 1. (a) Implant site of FAD-SA (50:50) polymer in brain 6 days after surgery. A well demarcated inflammatory response consisting of a small area of acellular necrosis with associated collection of neutrophils undergoing karyorrhexis surrounding the polymer; H & E 20×. (b) Implant site of FAD-SA (50:50) polymer 36 days after surgery. Localized areas of inflammation surrounding the polymer with associated macrophages; H & E 20×.

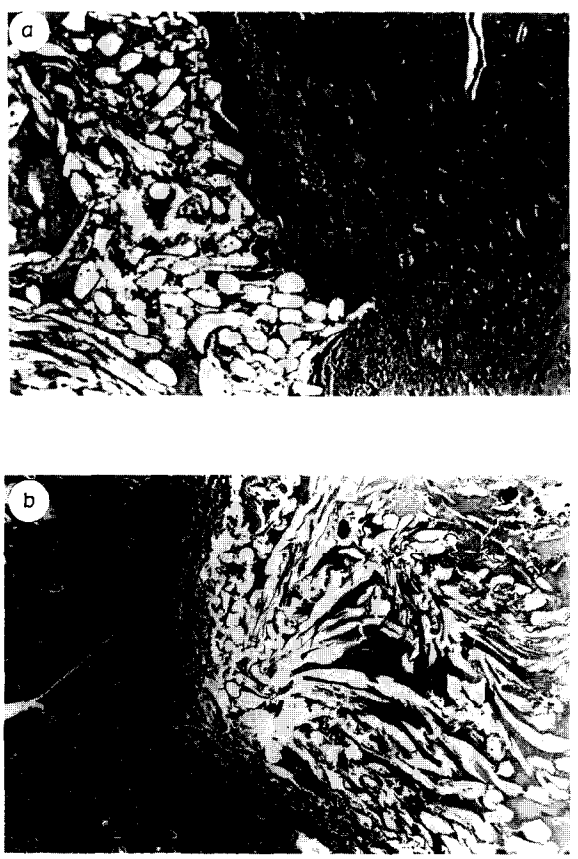

Fig. 2. (a) Implant site of Surgicel^{R} in brain 6 days after surgery. The Surgicel^{R} is surrounded by an area of acellular necrosis and lymphocytic inflammation; H & E 20×. (b) Surgicel^{R} 36 days after surgery. There is a mild area of chronic inflammation; H & E 20×.

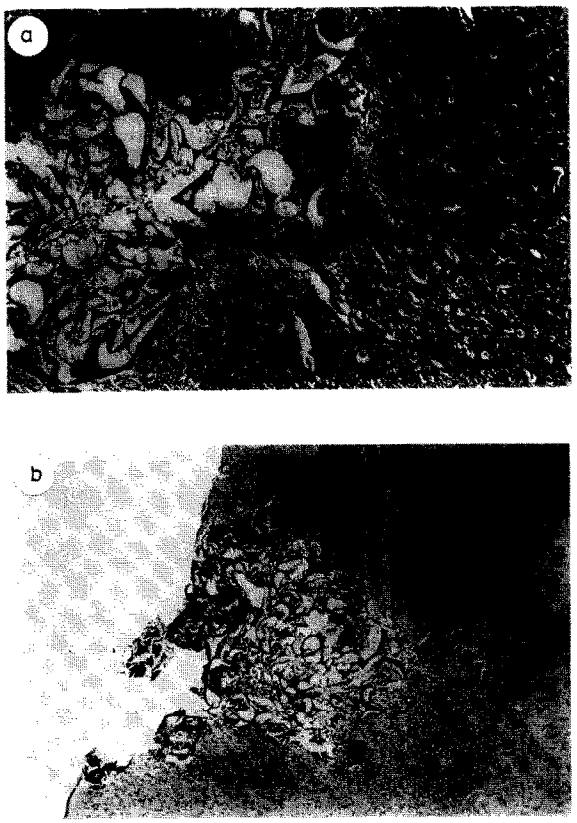

Fig. 3. Implant site of Gelfoam^R in brain 6 days after surgery. Gelfoam^R with minimal surrounding lymphocytic infiltrate and edema; H & E 20×. (b) Gelfoam^R 36 days after surgical implant with almost no surrounding tissue reaction; H & E 4×.

In the *chronic phase* (days 15–36) Surgicel^R was associated with the most pronounced fibrous response surrounding the implant (Fig. 2b), while p(FAD-SA) and Gelfoam^R had less chronic inflammatory responses (Fig. 1b and 3b). The p(FAD-SA) polymer was almost totally degenerated by day 36 with only hemosiderin laden macrophages as remnants of an inflammatory response.

Discussion

A number of biodegradable polymers have been developed during the past few years as a new class of carrier matrices for controlled release drug delivery systems [13]. Polyanhydrides have been extensively studied as vehicles for the release of bioactive molecules including drugs, peptides, and proteins. In particular the polyanhydride p(PCPP-SA) has been used, both experimentally and clinically, to deliver BCNU for the treatment of brain tumors [1,10].

In search for the optimal carrier for water soluble compounds, a new biodegradable anhydride has been developed. This is a copolymer of a fatty acid dimer (FAD), derived from the naturally occurring oleic acid and the sebacic acid p(FAD-SA). The primary advantage of this polymer is its excellent release kinetics for water soluble drugs. This is particularly important for delivery to the brain in that the blood-brain barrier effectively blocks most water soluble drugs from entering the brain substance.

In this report we have demonstrated that the p(FAD-SA) polymer delivery vehicle is biocompatible in the rat brain. The pattern of well demarcated acute inflammatory reaction is similar to that reported for the polyanhydride, poly [bis (p-carboxyphenoxy) propane-sebacic acid] copolymer (PCPP-SA) in the rat brain [14], rabbit brain [15], and monkey [1]. Based on these animal studies, a Phase I clinical trial was carried out utilizing PCPP-SA with BCNU to treat recurrent malignant brain tumors. As predicted from the experimental studies, there was no significant reaction to the polymers either clinically or pathologically when implanted in humans [10]. Currently, an ongoing placebo-controlled polymer implant clinical study will allow for the evaluation in humans of the polymers themselves without drug.

Based on the similarity of the reaction of the new fatty acid dimer with sebacic acid, p(FAD-SA), we would predict that this polyanhydride will be similarly useful clinically.

The localized inflammatory response generated by the p(FAD-SA) polymer is comparable to the response to Surgicel^R and previously tested polyanhydrides, but more pronounced than the reaction evoked by Gelfoam^R. This particular polymer is well suited for the interstitial delivery of water soluble drugs in the brain, thus offering the possibility to a number of bioactive com-

pounds to reach adequate concentrations in areas "protected" by the blood-brain barrier.

Acknowledgements

This work was supported by the NIH NCDDG UO1 CA52857, the Mellon Foundation and the Dana Foundation. The authors thank Michael Pinn and Carla Reinhard for technical assistance and Betty Collevecchio for preparation of the manuscript.

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