Clinical Materials **13** (1993) 163–168

Ocular and Brain Biocompatibility of Polymeric Delivery Materials Prepared from Ester Derivatives of Hyaluronic Acid

A. Olivi,[a] K. D. Kim,[a] L. Benedetti,[e] J. I. Epstein,[d] M. L. Pinn,[a] L. Callegaro[e] & H. Brem[a,b,c,*]

[a]Department of Neurosurgery, [b]Department of Ophthalmology, [c]Department of Oncology, [d]Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA. [e]Fidia S.p.A, Abano Terme, Italy

Abstract: We have tested the biocompatibility of a new class of controlled-release polymers derived from hyaluronic acid using two in-vivo models: the rabbit cornea and the rat brain. We compared the tissue reaction in the presence of these materials to that seen with absorbable gelatin sponge (Gelfoam^{R}) and oxidized regenerated cellulose (Surgicel^{R}).

In the cornea, the polymers produced different inflammatory responses, which generally correlated with their rate of degradation in the tissue. In the brain, similar degrees of inflammation and rates of degradation were observed, with no evidence of adverse neurological reaction or systemic toxicity. We conclude that polymers derived from hyaluronic acid should be explored further as potential vehicles for carrying therapeutic agents into the brain, and that the rabbit cornea and rat brain models could be used as an initial screen for polymer biocompatibility.

INTRODUCTION

Biodegradable polymers have recently been developed as a means of providing sustained release of therapeutic agents in target tissues, which produces high concentrations of the agent locally and relatively low levels in other organs.[1] This is of particular interest in the treatment of disorders of the central nervous system, where the blood–brain barrier limits access of drugs, and the high concentrations required systemically are often toxic. Therefore, a delivery system that circumvents the blood–brain barrier and provides large local concentrations of appropriate drugs is highly desirable.[2,3]

Although several types of polymer are available as vehicles for drug delivery, the search for novel forms continues, with the possibility of administering a wider variety of macromolecules. The polymers must have appropriate physical properties and release kinetics, and must be biocompatible with living tissues. In this context, polymers derived from hyaluronic acid have recently been developed.[4,5] In these products, the carboxyl groups of hyaluronic acid are esterified with therapeutically inactive and active alcohols, which produces polymers into which additional therapeutic agents can be incorporated. The release kinetics of hydrocortisone incorporated into different hyaluronic acid polymer preparations showed that they are suitable vehicles for drug delivery,[5] but their biocompatibility has not been fully tested. The present study was designed to determine the tissue compatibility of a series of hyaluronic acid polymers formed by esterification with a variety of compounds and different degrees of esterification.

Because we are interested in using the hyaluronic acid polymers for drug delivery in the brain, we chose two model systems for this study: rabbit cornea and rat brain. Implantation into the rabbit

* To whom correspondence should be addressed.

Clinical Materials 0267-6605/93/$6.00

cornea is relatively simple, and even minimal signs of inflammatory response can be observed and assessed.[6] The rat brain, on the other hand, would provide the initial indication of possible neurotoxicity of an intracerebrally implanted material as well as a qualitative estimate of the interaction between the biodegradable compound and the CNS tissue.[7,8] Using these two systems, we compared the tissue reactions of the hyaluronic acid derivatives with those of absorbable gelatin sponge (Gelfoam^R) and oxidized regenerated cellulose (Surgicel^R), two materials that are routinely implanted in the brain during neurosurgical procedures.

MATERIALS AND METHODS

Animals

All animal procedures were approved by the Johns Hopkins University Animal Care and Use Committee.

New Zealand White male rabbits weighing 2–3 kg were obtained from Bunnyville Farm (Littlestown, PA) and used for the cornea test. They were kept in standard animal facilities, one animal per cage, and given free access to food and water.

Fischer 344 adult male rats were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) and used for the brain assay. They were kept four per cage with free access to food and water.

Anesthesia

The rabbits were anesthetized by intramuscular injection of a mixture of xylazine 15–17 mg/kg and ketamine 15–17 mg/kg before the corneal implantation and before each stereomicroscopic examination of the cornea.

The rats were anesthetized with an intraperitoneal injection of 3–5 ml/kg of a stock solution containing ketamine 25 mg/ml, xylazine 2·5 mg/ml, and 14·25 % ethyl alcohol in normal saline.

Polymer implants

The polymeric materials were obtained from Fidia S.p.A. These esters were prepared as described in Table 1. As indicated, the polymers were provided in two different preparations; powder form and granular form. The powdered polymers were tested only in the cornea because they could not be implanted in the brain without dispersion in the subarachnoid space. The granular polymers were tested both in the rabbit cornea and in the rat brain. The materials were all sterilized by gamma-radiation (1·5 Mrads). Gelfoam^R was obtained in sterile packages from Upjohn, Kalamazoo, MI. Surgicel^R, also in sterile packages, was obtained from Johnson & Johnson, New Brunswick, NJ. At the time of the implant, sterility was confirmed by observing a fragment of the sample placed in blood agar culture plates for any subsequent bacterial growth.

Table 1. The polymers tested, (sodium hyaluronate (HA) esterified as shown)

Esterifying compound	*Percentage esterified*	*Designation*
Hydrocortisone	25	HYC 22
Methylprednisolone	30	HYC 33
Methylprednisolone	50	HYC 41
Methylprednisolone	85	HYC 85
Benzyl alcohol	25	HYAFF 11 p25
Benzyl alcohol	75	HYAFF 11 p75
Dodecyl alcohol	80	HYAFF 73 p80
Ethyl alcohol	100	HYAFF 7
Propyl alcohol	100	HYAFF 9
Benzyl alcohol	100	HYAFF 11

Since hyaluronic acid is a polycarboxylic acid, its esters may be prepared using standard methods for the esterification of carboxylic acids, such as the treatment of free hyaluronic acid with the desired alcohols in the presence of appropriate catalysts.

In more detail, our esters are prepared by treating a quaternary ammonium salt of hyaluronic acid with an esterifying agent in a suitable aprotic solvent at temperatures between 0° and 100° (Hyaff series), preferably 25°.

The hyaluronic acid used was a purified preparation from rooster's comb. This was a mixture comprising molecular fractions of the integral acid with molecular weights varying within a range from 100 000 to 300 000 Daltons. Tetrabutylammonium salts of hyaluronic acid were prepared by percolating sodium or potassium hyaluronate in aqueous solution through a salinized sulphonic resin in the tetrabutylammonium form and by freeze-drying the eluate. These salts have proved to be soluble in organic solvents. The esterification was carried out by gradually adding the alkylating agent to the tetraalkylammonium salt in dimethylsulfoxide (DMSO). Using this original synthesis, the number of carboxylic groups of the hyaluronic acid which are to be esterified can be precisely determined.[10]

Cornea assay

Each polymer was implanted into four rabbit corneas as previously described.[6] Briefly, after the rabbit was anesthetized, the cornea was irrigated with Proparacaine hydrochloride 0·5% (Allergan Pharmaceutical Inc., Irvine, CA) for local anesthe-

sia. Through a central cornea half-thickness incision, a radial pocket was created by using a cyclodialysis spatula. The bottom of the pocket was about 2 mm from the limbus. The granular material, Gelfoam^R, and Surgicel^R were cut into 1 mm^3 pieces and inserted into the bottom of the pocket. For powdered polymers, the bottom of the pocket was filled with the material to a height of about 1 mm. The cornea was again irrigated with Proparacaine hydrochloride 0·5%. The corneas were examined 7, 14, and 21 days after the implant with a slit lamp microscope (Zeiss Inc., New York, USA). The degree of degradation of the polymer was also assessed by direct visual inspection. Evidence of inflammatory response in the form of neovascularization, edema, or opacification of the cornea was recorded. Photographs of each cornea were taken at the time of the examination.

Brain implantation technique

Twenty-four rats were randomly divided into four groups of six. In each group, three polymers (HYAFF 7, HYAFF 9, HYAFF 11), Surgicel^R, and Gelfoam^R were implanted as follows: Part of the scalp was shaved and cleaned with 70% ethyl alcohol and Prepodyne solution. A midline incision was made and the sagittal and coronal sutures were identified. Bilateral burr holes 3 mm in diameter were placed just behind the coronal suture and 2 to 3 mm from the midline. The dura was exposed and an incision 3 mm in depth in an avascular region of the cortex was made with a #11 blade. The implants were introduced into the cortical defect. In a randomized manner, each of the granular polymers was implanted in one hemisphere and either Gelfoam^R or Surgicel^R was implanted in the other. The materials were alternately implanted in the right and left hemisphere to provide internal controls for the effects of differences in the sites of implantation. The size of the implants was always 1 mm^3. The skin of the scalp was then closed with surgical staples. The animals were returned to their cages and allowed to recover. The rats were examined twice daily after surgery for development of any neurologic deficit or behavioral change. The animals were sacrificed with an overdose of ether on postoperative days 3, 9, 21, and 36. The brains were removed and fixed in 10% phosphate-buffered formalin. The specimens were then cut coronally, embedded in paraffin, sectioned, and stained with Hematoxylin and Eosin. The inflammatory reaction in the region of the implants was assessed by light microscopy. Generalized features of the inflammatory response, such as necrosis, edema, gliosis, and hemorrhage were described. The histological appearance of the implants undergoing degradation was also examined.

Table 2. Cornea test results

Materials	*Corneal inflammation*	*Degradation time*
HA	–	< 7 days
HYC 22	–	< 7 days
HYC 33	–	< 7 days
HYC 41	–	7–14 days
HYC 85	–	14–21 days
HYAFF 11 p25	–	< 7 days
HYAFF 11 p75	+	14–21 days
HYAFF 73 p80	+	14–21 days
HYAFF 7	++	21–28 days
HYAFF 9	++	21–28 days
HYAFF 11	+	21–28 days
Gelfoam^R	–	7–14 days
Surgical^R	–	7–14 days

The material tested was considered inert (–) when no inflammatory reaction was observed in any of the four corneas receiving the implant. The material was considered moderately inflammatory (+) when one or two corneas out of four showed inflammatory changes in the form of neovascularization and edema. The material was considered markedly inflammatory (++) when a consistent and pronounced inflammatory reaction was observed in at least three of the four corneas on all the observation days.

RESULTS

Rabbit cornea assay

Table 2 summarizes the cornea test results, including the observed degradation time. Gelfoam^R and Surgicel^R, as well as the powdered hyaluronic acid derivatives HYC 22, 33, 41, 85, and HYAFF 11 p25 did not cause any inflammation in the cornea and were considered inert (Fig. 1). HYAFF 11, HYAFF 11 p75, and HYAFF 73 p80 showed moderate inflammatory responses, whereas HYAFF 7 and HYAFF 9 showed consistent and marked inflammatory responses in the cornea (Fig. 2). We also observed the degree of degradation of these materials in the cornea. Generally, the implants that were degraded rapidly (within 1 week), such as the HYC polymers, Gelfoam^R, and Surgicel^R, also showed no inflammatory response in the cornea, whereas the materials that had a degradation time of 2–3 weeks showed an inflammatory response. For example, of the three poly-

Fig. 1. Stereomicroscopic photograph of a non-inflammatory polymer (HYC 41) in the rabbit cornea 7 days after implantation. Traces of the degrading polymer are seen (arrow) but no inflammation is noted. The cornea is clear and avascular.

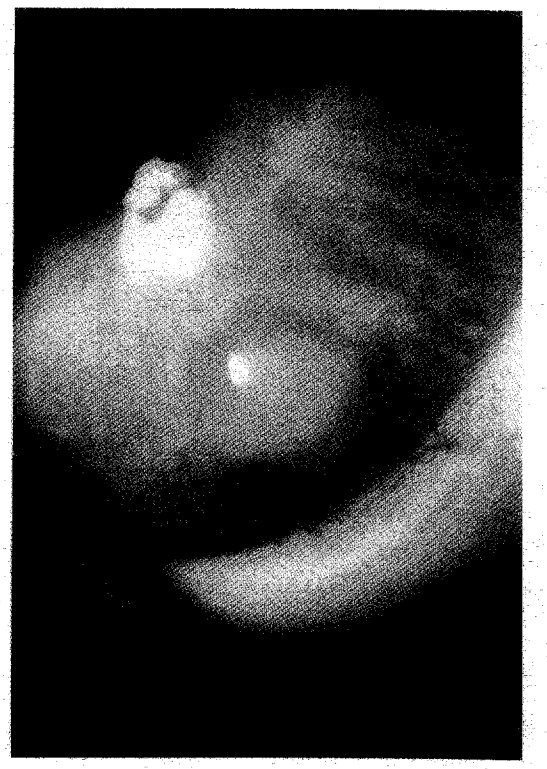

Fig. 2. Steromicroscopic photograph of an inflammatory polymer (HYAFF 7) in the rabbit cornea 7 days after implantation. The polymer is clearly visible at the bottom of the corneal pouch. A number of new vessels is filling the gap between the limbus and the polymer.

mers obtained in granular form, HYAFF 7 and HYAFF 9 showed marked inflammatory response, while HYAFF 11 showed a delayed, less pronounced but still observable reaction in the form of neovascularization of the cornea.

Rat brain

None of the animals undergoing bilateral intracerebral implant showed behavioral changes or neurologic deficits suggestive of either systemic or localized toxicity from the implants. All the animals survived to the scheduled date of sacrifice. From the histological standpoint, inflammatory changes around the implant were recorded based on the presence of focal necrosis, degenerating polymorphonuclear leukocytes, edema in the acute phase (3–9 days post-implant), and the presence of the histiocytic and lymphocytic infiltrates and gliosis in the subacute (9–21 days post-implant) and chronic phase (21–36 days post-implant).

On day 3 after surgical implantation, Surgicel^{R} evoked a pronounced inflammatory response, characterized by a rim of surrounding necrotic brain tissue and mild edema. This reaction, however, appeared to be fairly localized (Fig. 3). The polymers HYAFF 7, 9, and 11 showed a similar acute reaction characterized by focal necrosis and some inflammatory infiltration (mostly polymorphonuclear leukocytes). While the degree of necrosis was comparable with that generated by Surgicel^{R}, in some specimens the extent of the inflammatory reaction was wider and not as well limited as with Surgicel^{R}. Gelfoam^{R} showed the mildest reaction with only minimal necrotic brain tissue surrounding the brain implant and minimal inflammatory infiltrate.

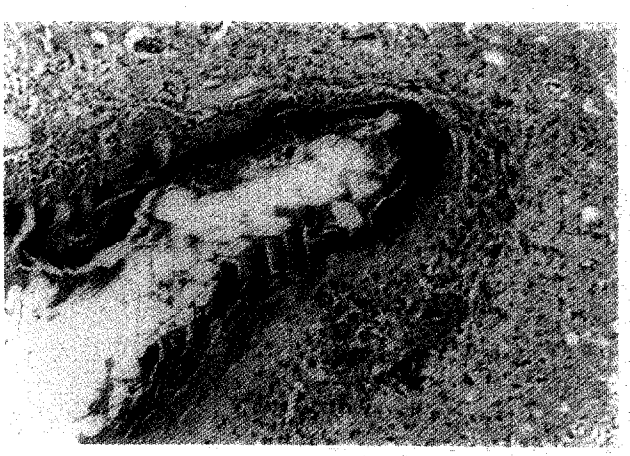

Fig. 3. Microphotograph of rat brain 3 days after surgical implantation of Surgicel^{R}. Note the rim of necrotic tissue around the implant with a localized inflammatory infiltration (mostly polymorphonuclear leukocytes). (Original magnification ×10.)

Fig. 4. Microphotograph of rat brain 6 days after surgical implantation of the polymer HYAFF 7. The implant is surrounded by a localized reaction characterized by a small rim of necrosis, histiocytic infiltrates and edema. In some areas (not shown) this reaction appeared to extend deeper than with Surgicel^{R} or Gelfoam^{R}. (Original magnification ×10.)

On day 6 after the implantation, a similarly prominent and localized reaction was noted around Surgicel^{R}. The histiocytic component was more evident at this time. There was still a zone of necrosis between the Surgicel^{R} and the inflammatory reaction. The three polymers tested again showed a more variable extent of inflammation, with some involvement at a greater distance from the implant when compared with the Surgicel^{R} (Fig. 4). The Gelfoam^{R} elicited only a modest inflammatory reaction.

On day 21, the three polymers had only a minimal inflammatory reaction in the form of edema and gliosis. Surgicel^{R} showed a similarly localized reaction, minimal edema, and some new growth of vessels. Gelfoam^{R} showed only minimal surrounding edema. At this time all the materials appeared to have degenerated and in some cases started to disappear from the implantation site.

On day 36, very little reaction to the material was noted in any of the specimens.

DISCUSSION

Biodegradable polymers prepared by esterification of hyaluronic acid with various steroids and alcohols have been shown to have rates of drug release *in vitro* that appear to be appropriate for their potential use as vehicles for drug delivery *in vivo*.[4,5] We have tested a variety of these polymers for compatibility with tissue in rabbit cornea and rat brain. We found that the polymers produced different inflammatory responses, which in general corresponded with their rates of degradation in the tissues. None of the polymers tested, however, generated any adverse neurological reaction or systemic toxicity, and even the more marked inflammatory reactions cleared relatively rapidly.

In this study we compared the inflammatory responses of the new materials with those generated by Surgicel^{R} and Gelfoam^{R}, which are used commonly for local hemostasis in neurosurgical procedures.[9] These materials showed a negligible response in the cornea and a limited local inflammation in the brain. The response of the cornea to the hyaluronic acid derivatives varied from negligible (HA, HYC 22, HYC 33, HYC 41, HYC 85, HYAFF 11 p25) to marked (HYAFF 7, HYAFF 9), with HYAFF 11 p75, HYAFF 73 p80, and HYAFF 11 showing moderate inflammation. In general , these responses correlated with the degree of degradation of the polymers: those that were degraded more rapidly produced a more modest response. For those polymers that were also tested in rat brain (HYAFF 7, 9, and 11), similar degrees of inflammation and rates of degradation were observed, thereby validating the usefulness of the two models for testing biocompatibility.

Despite the marked inflammatory response to polymers HYAFF 7 and 9, no concomitant adverse neurological or systemic reactions were observed in the rats receiving the brain implants. This suggests that these and other polymers should be explored further as potential vehicles for carrying therapeutic agents into the brain. Modifications in the structure of the polymers with appropriate selection of esterifying compounds and percentages could further improve the tissue compatibility of these materials without affecting their physical properties as drug carriers. Our two in-vivo models could be used in the future to confirm the improved biocompatibility.

REFERENCES

1. Langer, R., New methods of drug delivery. *Science*, **249**, (1990) 1527–33.
2. Brem, H., Mahaley, M. S., Vick, N. A., Black, K., Schold, S. C., Burger, P. C., Friedman, A. H., Ciric, I. S., Eller, T. W., Cozzens, J. W. & Kenealy, J. N., Interstitial chemotherapy with drug polymer implants for the treatment of recurrent gliomas. *J. Neurosurg.*, **74** (1991) 441–6.
3. Brem, H., Polymers to treat brain tumors. *Biomaterials*, **11** (1990) 699–701.
4. Hunt, J. A., Joshi, H. N., Stella, V. J. & Topp, E. M., Diffusion and drug release in polymer films prepared from ester derivatives of hyaluronic acid. *J. Controlled Release*, **12** (1990) 159–69.

5. Benedetti, L. M., Topp, E. M. & Stella, V. J., Microsphere of hyaluronic acid esters — Fabrication methods and in-vitro hydrocortisone release. *J. Controlled Release*, **13** (1990) 33–41.
6. Langer, R., Brem, H. & Tapper, D., Biocompatibility of polymeric delivery systems for macromolecules. *J. Biomed. Mater. Res.*, **15** (1981) 267–77.
7. Tamargo, R. J., Epstein, J. I., Reinhard, C. S., Chasin, M. & Brem, H., Brain biocompatibility of a biodegradable, controlled-release polymer in rats. *J. Biomed. Mater. Res.*, **23** (1989) 253–66.
8. Brem, H., Kader, A., Epstein, J. I., Tamargo, R. J., Domb, A., Langer, R. & Leong, K. W., Biocompatibility of a biodegradable, controlled-release polymer in the rabbit brain. *Selective Cancer Therapeutics*, **5** (1989) 55–65.
9. Arand, A. G. & Sawaya, R., Intraoperative chemical hemostasis in neurosurgery. *Neurosurgery*, **18** (1986) 223–33.