Biodegradable tube implants in experimental glaucoma surgery in the rabbit

M. KIVALO, V. SIRÉN*, C. RAITTA‡, I. IMMONEN‡
*Department of Basic Veterinary Sciences, *Department of Virology, and ‡Department of Ophthalmology, University of Helsinki, Helsinki, Finland*

Although ocular drainage implants are manufactured from biocombatible materials to reduce foreign-body reaction, the formation of excessive scar tissue around the implant is a common cause for implant failure. In this study, the suitability of poly(*D*, *L*-lactide-co-glycolide) copolymer, impregnated with an antiproliferative agent retinoic acid, was evaluated as a material for biodegradable tubular implants, as well as the duration and magnitude of the intraocular pressure reduction obtained with the prototype implant. Subconjunctivally placed retinoid-impregnated polymer particles caused a milder inflammatory reaction than plain polymer, and the layer of connective tissue around the material was thinner after the follow-up period of 60 d. In the anterior chamber, the inflammatory response elicited by the material was milder than subconjunctivally. The plain polymer caused a transiently stronger reaction than the retinoid-impregnated polymer, but after 60 d no difference was evident between the two materials. In all operated eyes with the tubular implant, the intraocular pressure was statistically significantly lower ($p < 0.05$) than in control eyes for 9 wk after the operation. The intraocular pressure of the eyes with the retinoid-impregnated implant was statistically significantly lower ($p < 0.05$) than in eyes with a plain polymer implant for up to 7 wk post-operatively. However, the use of retinoid did not prolong the effective functioning time of the implants.

1. Introduction

Successful outcome of glaucoma filtering surgery depends on the formation of a functioning filtration bleb, with excessive scar tissue formation in the subconjunctival space being a common cause for bleb failure. By use of ocular drainage implants from biocompatible materials such as polymethylmetacrylate [1] and silicone [2], combined with anti-inflammatory medication [3] and antimetabolites 5-fluorouracil [4] or mitomycin-c [5] inflammation, foreign-body reaction and subsequent fibrosis can be reduced.

Ocular surface toxicity of the antimetabolites and discomfort of the frequent administrations have, however, led to a search for different drug-delivery systems. Alvarado [6] used liposomes, Hasty *et al.* [7] collagen implants, and Lee *et al.* [8] biodegradable materials as carrier vehicles for the drugs, with prolonged bleb life and fewer side effects reported.

Retinoids, synthetic derivatives of vitamin A, are known to be able to affect connective tissue metabolism [9] and modulate the function of inflammatory cells [10]. They have also been reported to have anti-inflammatory effects [11].

In our work, we combined the use of a filtration device and an antiproliferative agent [12] by manufacturing the tubular implant from poly (*D*, *L*-lactide-co-glycolide) (PGA/PDLLA) co-polymer impregnated with retinoic acid. A biodegradable implant allows continuous filtration of anterior chamber fluid into the subconjunctival space during the first months after surgery when the scar tissue formation is most active, and is later degraded, leaving a patent filtrating fistula.

Antiproliferative agents released by the implant will also inhibit excessive scar-tissue formation around the implant.

The aim of this study was two-fold: first, to evaluate the suitability of PGA/PDLLA-polymer as a material for ocular drainage implants and as a delivery vehicle for retinoid; and second, to evaluate the duration and magnitude of the intraocular pressure (IOP) reduction obtained with the prototype implant in the rabbit eye.

2. Materials and methods

2.1. Materials

The resorbable (biodegradable) copolymer, poly(*D*, *L*-lactide-co-glycolide) 50:50 came from Boehringer Ingelheim KG, Ingelheim, Germany. Polyglycolide (polyglycolic acid, PGA) and polylactide (polylactic acid, PLA) are synthetized by ring-opening polymerization of the cyclic diesters of glycolic and *D*, *L*-lactic acid [13]. Polyglycolide is a crystalline polymer, whereas poly(*D*, *L*-lactide) is amorphous. The molecular weight, M_w, of PGA/PDLLA is 115000, and the inherent viscosity 0.8 dl g^{-1}. Degradation time *in vivo* was about 2 mon.

All-*trans*-9-(4-methoxy-,2,3,6-trimethyl-phenyl)-3, 7-dimethyl-2,4,6,8-nonatetraenoic acid (Acitretin) was

provided by Hoffmann La Roche (F. Hoffmann La Roche Ltd, Basel, Switzerland). This retinoid was dissolved in ethanol at a concentration of 2 mM and was stored at -20° C; 0.5 g PGA/PDLLA polymer was dissolved in 1.5 ml chloroform with and without retinoid. The final concentration of retinoid in the chloroform solution was 10 μM. The solution with polymer was then processed into tubes (3 mm × 15 mm).

2.2. Operative procedure

Surgery was performed on 20 New Zealand White rabbits. For surgery, all animals were anaesthetized with a combination of xylazine hydrochloride (Rompun^{R} 20 mg ml^{-1}, Bayer, Leverkusen, Germany) 5 mg kg^{-1} and ketamine hydrochloride (Ketalar^{R} 50 mg ml^{-1}, Parke-Davis, S.A. Barcelona, Spain) 15 mg kg^{-1} intramuscularly. The cornea and conjunctiva were topically anaesthetized with oxybuprocaine hydrochloride (Oftan-Obucain^{R} 4 mg ml^{-1}, Leiras, Turku, Finland). With the aid of an operating microscope, two fornix-based subconjunctival pockets were made in the left eye of each of ten animals (group A), one in the upper temporal and one in the upper nasal quadrant. A piece of plain PGA/PDLLA-polymer was inserted in one and a piece of retinoid-impregnated polymer in the other pocket of each eye. The pockets were closed at the limbal incision with two single 8–0 Vicryl (Vicryl^{R}, Ethicon, Norderstedt, Germany) sutures.

In the right eyes of the same animals, either a piece of plain or of retinoid-containing PGA/PDLLA-polymer was inserted into the anterior chamber through a limbal incision. The incisions were closed with a continuous 8–0 Vicryl suture, and the anterior chambers were reformed with balanced salt solution (BSS, Alcon Laboratories Inc., Fort Worth, TX, USA).

In the remaining ten animals (group B) either a plain or a retinoid-containing PGA/PDLLA-polymer tube of 3 mm × 15 mm (five of each) was inserted into the anterior chamber of the right eye through a limbal incision. The caudal ends of the tubes were positioned episclerally about 10 mm from the limbus, and conjunctival flaps were sutured over the limbal incision with single 8–0 Vicryl sutures. The anterior chambers were reformed with physiological saline solution. The non-operated left eyes served as a control for the IOP values.

All animals received, as a systemic antibiotic, trimethoprim 12.5 mg ml^{-1} and sulphadoxin 62.5 mg ml^{-1} (Borgal^{R} mite vet, Hoechst, München, Germany) subcutaneously once a day for 4 d. Locally, chloramphenicol 2 mg ml^{-1} – hydrocortisone 5 mg ml^{-1} (Oftan C–C^{R}, Leiras, Tampere, Finland) eye emulsion and atropin 1% (Oftan-Atropin^{R}, Leiras) solution were administered three times a day for 4 wk.

2.3. Follow-up studies

Biomicroscopical examination of the anterior segments of all operated eyes and measurement of IOP were carried out daily for the first four post-operative days and then once a week for the rest of the follow-up period. A digilab Modular One Pneuma-Tonometer (Bio-Rad, Ophthalmic Division, Cambridge, MA, USA) was used for the IOP measurements.

Follow-up periods in group A were 5 d for two animals, 2 wk for two animals, 4 wk for two animals and 8 wk for four animals. In group B, two animals were monitored for 12 wk and eight animals for 16 wk.

After the follow-up period, the animals were euthanized and the eyes were enucleated and immediately fixed in 10% neutral phosphate-buffered formaline. For histological sectioning the eyes were embedded in paraffin, cut with a microtome into 5 μm thick sections and stained with haematoxylin–eosin.

Data from IOP measurements are expressed as mean ± S.E. of the subtraction values of pressures of the control and operated eyes (ΔP = IOP of control eye minus IOP of operated eye).

The statistical analyses were carried out with the repeated measurements analysis of variance in the general linear model procedure in SAS [14]. A difference was considered statistically significant when $p < 0.05$.

The use of research animals in this study was approved by the ethical committee of The Faculty of Veterinary Medicine, University of Helsinki.

3. Results

3.1. Group A

3.1.1. Subconjunctival PGA/PDLLA

3.1.1.1. *Biomicroscopy*. Eyes with subconjunctival implants showed, at the site of surgery, only a slight hyperaemia that resolved by the fourth post-operative day. No changes were seen in the anterior chambers or intraocular pressures.

3.1.1.2. *Histology*. At day 5, subconjunctivially placed plain polymer was surrounded by a layer of loose connective tissue with numerous fibroblasts and a large number of mononuclear leucocytes. Only a few polymorphonuclear leucocytes were visible.

A thinner layer of loose connective tissue surrounded the retinoid-containing implants, and also the number of mononuclear and polymorphonuclear leucocytes was smaller, especially at the border between the connective tissue and the polymer.

At 14 d post-operatively, the connective tissue surrounding the plain polymer had increased in thickness and density. Large numbers of both mononuclear and polymorphonuclear leucocytes were seen, especially at the borders of the polymer.

The retinoid-impregnated implant was surrounded by a layer of connective tissue slightly thicker and better organized than in the specimens taken at day 5. The number of mononuclear and polymorphonuclear leucocytes had somewhat increased, mononuclear cells being the predominant cell type. They were mainly visible in the vicinity of the implant.

At day 30, both implant types had decreased in size and broken into several smaller pieces. Surrounding connective tissue containing numerous mononuclear

leucocytes protruded also between the polymer fragments. Clusters of large mononuclear cells (giant cell formation) were seen on the surface of the pieces. The reaction was stronger on the plain polymer.

At 60 d post-operatively, only small fragments of the implant material could be seen. The remains of the plain polymer were surrounded by dense, fairly unorganized connective tissue with large numbers of mononuclear cells and small capillaries. The connective tissue surrounding the retinoid-containing polymer was better organized, with only a small number of mononuclear leucocytes embedded in the remaining pieces of material.

3.1.2. Anterior chamber PGA/PDLLA

3.1.2.1. *Biomicroscopy*. All eyes with a piece of plain or retinoid-containing polymer in the anterior chamber showed mild signs of iritis post-operatively for the first few days. Fibrin had accumulated in the AC during the first post-operative day. The amount of fibrin seemed to be less in the retinoid group, in which it had disappeared from the anterior chamber during the first post-operative week. In the eyes with the plain polymer, the last of the fibrin had vanished by the end of the second week. Pieces of retinoid-impregnated polymer were visible on the anterior side of the iris maximally for 7 wk, until they had completely resorbed.

Plain PGA/PDLLA pieces were gradually being enveloped by iris tissue after the first week and were totally covered or resorbed in 6 wk.

3.1.2.2. *Histology*. For the eyes with the polymer in their anterior chamber, the first preparations were made 5 d after surgery. In these the retinoid-impregnated polymer granules were apparent on the anterior surface of the iris with some fibrin and solitary mononuclear and polymorphonuclear leucocytes between the material and iris tissue.

Particles of the plain polymer were partly embedded in the iris tissue with few mononuclear and polymorphonuclear leucocytes on the surface of the material. A thin layer of fibroblasts was covering part of the polymer surface.

At day 14 the retinoid-containing polymer was still clearly visible on the anterior surface of the iris (Fig. 1a). Iris tissue had started to cover it around the edges. Occasional mononuclear cells and fibroblasts were visible on the surface of the polymer.

The plain polymer (Fig. 1b) was almost completely covered by iris tissue. Increased connective tissue was apparent with numerous mononuclear and polymorphonuclear leucocytes around the material.

At 30 d post-operatively, iris tissue still covered only part of the retinoid-containing polymer (Fig. 2a), whereas an increased amount of connective tissue with mononuclear cells remained between the plain polymer (Fig. 2b) and the iris tissue. The number of mononuclear cells around the retinoid impregnated material was about the same as in the previous sample.

The remains of the retinoid-containing material at 60 d post-operatively, were small granules embedded in the anterior surface of the iris, surrounded by a thin layer of connective tissue with a few mononuclear leucocytes. The remains of the plain polymer did not differ from those of the retinoid-containing polymer in respect to the amount of connective tissue or mononuclear cells.

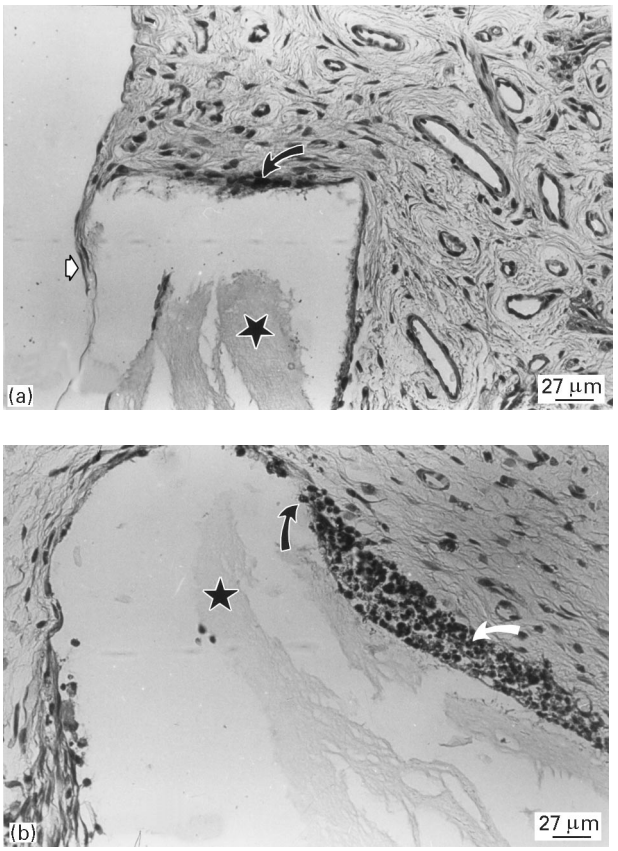

Figure 1 (a) Retinoid-impregnated and (b) plain PGA/PDLLA-polymer material (∗) on the anterior side of the iris 14 d post-operatively. Occasional mononuclear cells (black arrow) and fibroblasts (open arrow) are visible on the surface of the retinoid-containing polymer. The plain polymer is covered by iris tissue with an increased number of mononuclear cells (black arrow) and polymorphonuclear leucocytes (white arrow); (HE).

3.1.2.3. *Intraocular pressure*. In eyes with PGA/PDLLA polymer in the anterior chamber, the IOP decreased transiently after the operation but returned to the pre-operative level after the first post-operative week.

3.2. Group B (Tubular PGA/PDLLA implants)

3.2.1. Biomicroscopy

During the first post-operative day, fibrin had accumulated in the anterior chamber in all eyes, less in the eyes with a retinoid-containing tube. In all eyes, fibrin disappeared from the anterior chamber during the first post-operative week. Signs of iritis, apparent mainly as congestion of iris capillaries, occurred after the operation in all eyes. These signs persisted from 2–3 wk, except in two animals (one with a plain and one with a retinoid tube) in which the last signs disappeared only by the end of the fourth week.

Plain implants showed the first signs of resorption 6 d after the operation, at which time they were being enveloped by iris tissue. Two of the implants were

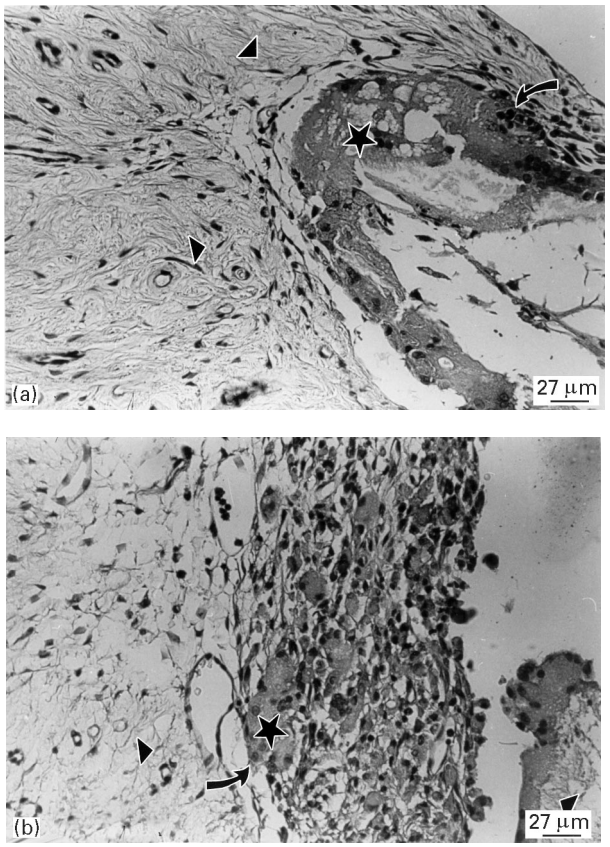

Figure 2 Mononuclear cells (black arrow) and iris tissue (arrow head) surrounding (a) the retinoid-impregnated and (b) plain polymer materials (*) 30 d post-operatively. Increased amount of connective tissue is seen among the particles of the plain polymer in (HE).

totally resorbed after 7 wk, two after 10 wk and one after 15 wk. Implants with retinoid-impregnated PGA/PDLLA-polymer began to be covered by iris tissue 6 d post-operatively. The first signs of resorbtion appeared 2 (one animal) to 4 (four animals) wk after the operation. Two of the implants were completely resorbed after 10 and 12 wk, the remains of the rest were still visible in the anterior chamber at 15 wk.

3.2.2. Histology

The first histological specimens (two animals) were obtained 12 wk after the operation. Unorganized granulation tissue occupied the sites in the cornea where the polymer tubes had been inserted into the anterior chamber. Small granules of the implant material could be seen among the collagen bundles. Larger amounts of the material were found as streaks caudal to the limbal incision, and were surrounded by a thin layer of dense organized connective tissue. A small number of mononuclear cells were scattered among the polymer granules, less on the retinoid-treated polymer than on the plain polymer. In all preparations, iris tissue had adhered to the edges of the limbal incisions and partly protruded into the wound channel, and were blending with the scar tissue.

The filtration angle of the eye with the plain PGA/PDLLA-tube contained numerous mononuclear cells and minute particles of the implant material.

The last of the histological specimens (eight animals) were made 16 wk after the operation. Of the four eyes with the retinoid-containing polymer, three had unorganized scar tissue blocking the channel in the cornea. In the remaining eye, a channel had formed through the cornea, ending at the limbus. In all specimens, adhesions had formed between iris and cornea at the site of the operation, with part of the iris tissue protruding into the corneal channel in two eyes.

Accumulations of the implant material (Fig. 3a), surrounded by a thin layer of organized connective tissue with numerous small capillaries, were seen subconjunctivally along the length of the resorbed polymer tube. The number of mononuclear cells among the implant material was smaller than in the eyes with the plain implant. Traces of the implant material and mononuclear cells were seen in the anterior chambers of two eyes.

In all eyes with the plain tube, at the site of the anterior chamber entry was an area of unorganized connective tissue into which iris tissue protruded in three specimens. Remains of the implant material (Fig. 3b) were seen subconjunctivally as separate clusters. They were surrounded by a thin layer of organized connective tissue with small capillaries and some mononuclear cells. In three eyes, mononuclear cells and implant material was visible in the anterior chamber.

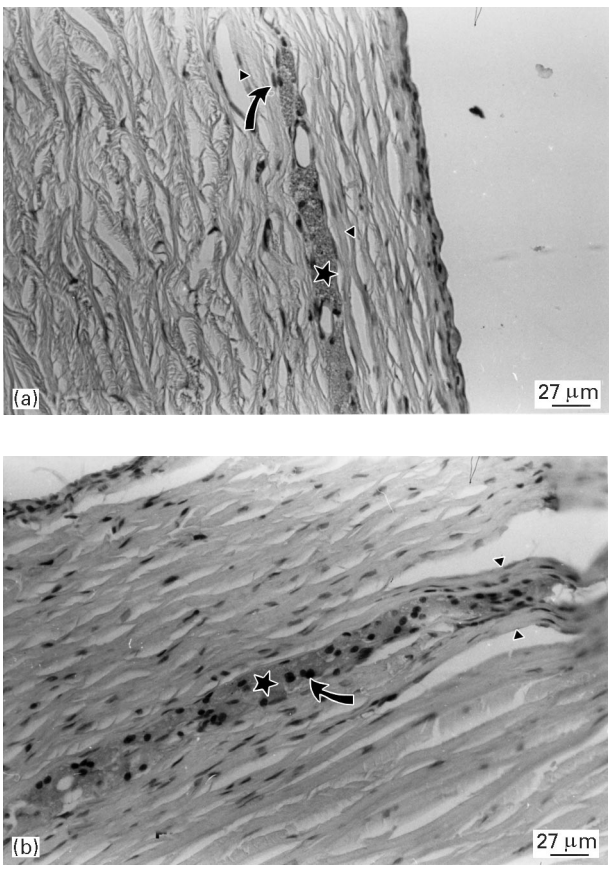

Figure 3 Remains of (a) the retinoid-impregnated and (b) plain tubular PGA/ PDLLA implants (*) surrounded by a thin layer of organized connective tissue (arrow head) 16 wk after the operation. Mononuclear cells (black arrow) are visible among the implant material; (HE).

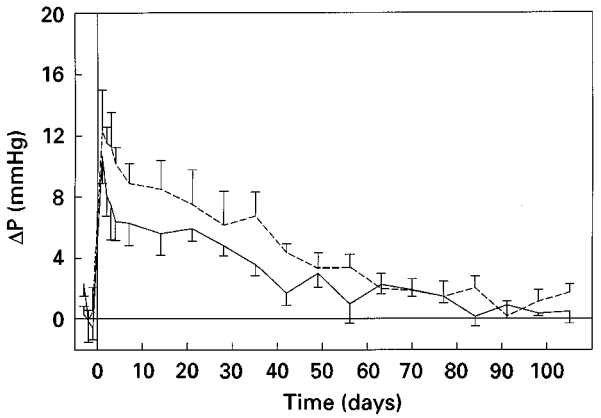

Figure 4 Results of the IOP measurements presented as mean ± S.E. of the subtraction values of pressure of the control and operated eyes. $\triangle P$ = IOP of control eye minus IOP of operated eye. (- - -) $\neq \triangle P$-retinoid, (——) $\neq \triangle P$-plain.

3.2.3. Intraocular pressure

Results of the IOP measurements are presented in Fig. 4 as a difference in pressure (ΔP-plain and ΔP-retinoid) between control eyes and operated eyes. The effect of the operation on IOP diminished over time, but in all operated eyes the IOP was statistically significantly lower ($p < 0.05$) than in control eyes for 9 wk after the operation.

The IOP was statistically significantly ($p < 0.05$) lower in eyes with the retinoid-containing tubes than in eyes with plain PGA/PDLLA tubes for up to 7 wk post-operatively. However, the use of retinoid did not prolong the effective functioning time of the implants.

4. Discussion

Biodegradable polymers have seen increasing use especially in orthopaedic surgery [15, 16] in recent years. The two most commonly used polymers are polyglycolic acid (PGA) or polylactic acid (PLA). These are degraded by hydrolysis into lactic acid, which is incorporated in the citric acid cycle and further broken down into carbon dioxide and water.

PGA and PLA have been found to be biocompatible both in human and in veterinary surgery. Cutright and co-workers [17–19] have described the tissue reactions of absorption of PLA. An inflammatory reaction with polymorphonuclear and mononuclear leucocytes is seen first, and later proliferation of fibroblasts in the surrounding connective tissue gradually replaces the degrading implant. The inflammatory reaction decreases with time [20], as the degradation of the implant proceeds.

In this work, the subconjunctivally placed polymer particles (group A) caused an inflammatory reaction shown by the accumulation of polymorphonuclear and mononuclear leucocytes on the material. The reaction was somewhat more evident around the plain PGA/PDLLA-polymer than around the retinoid-impregnated polymer during the whole follow-up period. This is in accordance with Bauer *et al.* [21] who reported inhibition of the migration of neutrophils by retinoid. In the retinoid group, a layer of connective tissue, that encapsulated the material as a result of the reaction, was thinner and more organized. Araiz *et al.* [12] reported the antiproliferative effect of retinoic acid on fibroblasts in an animal model of proliferative vitreoretinopathy. Retinoic acid has also been reported to inhibit production of collagen by human skin fibroblasts [22] and human lung fibroblasts [9].

In the anterior chamber, the inflammatory response elicited by the polymer particles was milder than subconjunctivally. The retinoid-impregnated implant was especially well tolerated, judged by the number of inflammatory cells and the amount of connective tissue in the histological sections. The plain polymer caused a transiently stronger reaction, but at the end of the follow-up period no difference was evident between the two materials.

Accumulation of fibrin and signs of iritis followed the insertion of the tubular implants into the anterior chamber. The amount of fibrin was less in the retinoid group. This was probably a consequence of the anti-inflammatory effect [10] of retinoids, which, according to Ney *et al.* [23], is a result of inhibition of interleukin-1-stimulated production of prostaglandin E_2.

In histological sections at the end of the follow-up period of 16 wk, only one eye showed an open channel through the cornea at the site where the retinoid-impregnated tube had been. The channel ended at the limbus, with no visible bleb. The IOP had also returned to the pre-operative level.

In an experimental study, Miller *et al.* [24] showed that sclerostomies in the rabbit were blocked by scar tissue by post-operative day 17. Similar results were obtained by Desjardins *et al.* [25], who reported that, after filtering surgery in owl monkeys, closing of the limbal fistulas occurred by day 14 post-operatively. In our experiment, the IOP was decreased for up to 9 wk after surgery, indicating that in the rabbit the implant greatly prolongs the time of filtration.

By use of products capable of inhibiting fibroblast proliferation and subsequent formation of scar tissue, the patency of sclerostomies and functioning of the filtration blebs can be prolonged. Because wound healing occurs over an extended time, use of topical or subconjunctival application does not provide an optimal antiproliferative effect for a long enough time period. Alternative delivery systems for these drugs are therefore needed to minimize side effects and provide better results in controlling fibroblast proliferation.

Lee *et al.* [8] used a seton made of a bioerodible polymer as a carrier for 5-fluorouracil. The solid cylindrical seton was placed in the sclerostomy opening where it would release the drug over a longer period of time. Although scar tissue eventually closed the fistula, filtration blebs lasted longer than in control eyes. However, bleb failures occurred in all animals before the fifth post-operative week, earlier than observed in our experiment.

Other studies with different drug delivery systems were made by Alvarado [6], who used liposomes as a carrier vehicle for the antimetabolite 5-fluoroorotate. Hasty *et al.* [7] manufactured collagen

implants with 5-fluorouracil, but found that the implant material caused a granulomatous inflammatory reaction. Charles *et al.* [26] experimented with a bioerodible polymer impregnated with mitomycin, and reported it to promote the success of glaucoma-filtration surgery. Polyanhydride discs containing the antiproliferative agents Taxol and VP-16, were used by Jampel *et al.* [27, 28] to inhibit fibroblast proliferation. Decrease in episcleral fibrosis in the rabbit was achieved by Strauss *et al.* [29] with subconjunctival plasminogen activator in an inert gel-delivery vehicle. A collagen sponge, containing either 5-fluorouracil or bleomycin, was used experimentally by Kay *et al.* [30] to prolong bleb life, and by Herschler [31] in clinical cases with good results in long-term follow-up. Injectable PLA microspheres that provided a controlled release of the antimetabolic agent adriamycin, were reported by Kimura *et al.* [32] to lower the IOP in the rabbit and prolong bleb function. Uppal *et al.* [33] used a bioerodible drug-carrier to release etoposide subconjunctivally for 12 d to reduce fibroblast proliferation.

The polymer implant in this study was tubular instead of the solid type used in other studies. This allowed it to function as a seton and mechanically maintain patency of the filtration site while releasing retinoid over the extended period of the wound-healing process. Because the implant finally dissolves, foreign-body reaction and implant erosion through the conjunctiva, which are common long-term complications with non-degradable implants, can be avoided. Ideally, the biodegradable implant would function as a template that could control, by means of sustained release of antifibrinolytic agents, the developing connective tissue, so that a patent fistula and a functioning filtration bleb can be maintained. The difference in pressure between operated eyes and control eyes in this study remained at a statistically significantly lower level for 9 wk post-operatively. Impregnating the implant with retinoid kept the IOP significantly lower for 7 wk compared to the case with tubes of the plain polymer. This may be due to the thinner fibrous tissue seen around the material. Possibly the amount of retinoid was too small to sustain the difference for longer.

Even though the fistulating surgery eventually failed, the results using PGA/PDLLA-polymer as an implant material and as a delivery vehicle for retinoid, seem promising in controlling fibrosis. By modulating the polymer to lengthen degradation time and altering the amount of retinoid, more long-lasting results could be possible.

Acknowledgments

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