Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 352 (2008) 240–247
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

A biodegradable drug delivery system for the treatment of postoperative inflammation

S. Eperon [a,*], L. Bossy-Nobs [b,1], I.K. Petropoulos [a,2], R. Gurny [b], Y. Guex-Crosier [a,**]

[a] *Jules Gonin Eye Hospital, Lausanne University, 15, Avenue de France, 1000 Lausanne 7, Switzerland*
[b] *Department of Pharmaceutics and Biopharmaceutics, School of Pharmaceutical Sciences, University of Geneva, 30, Quai E.-Ansermet, 1211 Geneva 4, Switzerland*

Received 17 July 2007; received in revised form 30 October 2007; accepted 31 October 2007
Available online 17 November 2007

Abstract

Cataract surgery is often performed in patients suffering from associated pathologies. Our goal is to develop a biodegradable drug delivery system (DDS) combined with the artificial intraocular lens (IOL). DDS were manufactured using poly(D,L-lactide-*co*-glycolide), or PLGA, and were loaded with triamcinolone acetonide (TA). The loading capacity was approximately 1050 μg of TA per DDS. The higher the molecular weight of PLGA (34,000, 48,000 and 80,000 Da), the slower was the release of TA in vitro. Cataract surgery was performed on the right eye of rabbits. IOL was inserted with (i) no DDS, (ii) unloaded DDS PLGA48000, (iii) one loaded DDS PLGA48000, (iv) two loaded DDS. The number of inflammatory cells and the protein concentration were measured in the aqueous humor (AH). Unloaded DDS showed good ocular biocompatibility. One DDS PLGA48000 loaded with TA significantly reduced postoperative ocular inflammation. Two loaded DDS PLGA48000 was even more effective in inhibiting such inflammation. On long-term observation (days 63 and 84), reduction of inflammation could be obtained by insertion of one DDS PLGA48000 and a second DDS PLGA80000. Therefore, our "all in one" system is very promising since it could replace oral treatment and reduce the number of intraocular injections.

Keywords: Biodegradable polymer; Drug delivery; Intraocular lens; Rabbit; Triamcinolone

1. Introduction

Cataract extraction is one of the most frequently performed surgeries in the world today. Each year about 2 million people have their cataracts removed and replaced with an intraocular lens IOL (Hankinson, 2000). Surgical trauma may induce the onset of cystoid macular edema (Wright et al., 1988; Miyake et al., 2000; Simone and Whitacre, 2001). Moreover, cataract surgery is often performed in older patients suffering from combined ocular pathologies such as uveitis, age-related macular degeneration (AMD) or proliferative diabetic retinopathy. Surgery can cause these pathologies to deteriorate and may lead to permanent visual loss. In AMD, ocular surgery may be followed by a rapid progression of choroidal neovascularization associated with neovessel proliferation (Adamis and Shima, 2005). Non-phakic eyes have been shown to develop a higher risk (odd ratio = 5.7) of late-stage maculopathy lesions compared to phakic eyes (Wang et al., 2003).

To prevent short- and long-term complications, corticosteroids and nonsteroidal anti-inflammatory drugs are applied topically in cataract surgery. In the presence of macular edema, systemic or local steroid injections have been used (Antcliff et al., 2001; El Harazi and Feldman, 2001; Simone and Whitacre, 2001; Eyetech Study Group, 2003). Simultaneous administration of anti-vasoproliferative agents (anti-VEGF) has also been suggested in AMD (Eyetech Study Group, 2003). However, the efficacy of these treatments is limited either by the blood–ocular

* Corresponding author. Tel.: +41 21 626 8259; fax: +41 21 626 8122.
** Corresponding author. Tel.: +41 21 626 8595; fax: +41 21 626 8122.
E-mail addresses: Simone.eperon@ophtal.vd.ch (S. Eperon), yan.guex@ophtal.vd.ch (Y. Guex-Crosier).
[1] Present address: TRB Chemedica International SA, rue Michel-Servet 12, 1211 Geneva 12, Switzerland.
[2] Present address: Division of Ophthalmology, University Hospital, rue Micheli-du-Crest 24, 1211 Geneva 14, Switzerland.

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.10.054

barriers (McGhee et al., 2002), poor drug bioavailability or serious side-effects. Intravitreal injections must be repeated to maintain the drug level within a therapeutic range, with the risks of vitreous hemorrhage, retinal detachment or endophthalmitis (Parke, 2003).

Biodegradable drug delivery systems (DDS) open a new route to control postoperative inflammation (Cahill and Jaffe, 2006). DDS are designed to increase drug bioavailability and effectiveness, prolong the controlled release of the drug and reduce systemic side-effects (Tan et al., 1999). Biodegradable DDS eliminates the need for a second surgery to remove the implant. Various solid DDS releasing drug into the posterior part of the eye have been investigated (Kunou et al., 2000; Okabe et al., 2003; Yasukawa et al., 2005). A few DDS have been developed to specifically treat inflammation following cataract surgery (Chang et al., 1999; Tan et al., 2001; Wadood et al., 2004): the Surodex® steroid DDS (Oculex Pharmaceuticals, Inc., Sunnyvale, CA) is placed in either the posterior or the anterior chamber at the conclusion of surgery. This device presents major disadvantages such as migration, discomfort, and the persistence of residues of the device after several months. The Posurdex® (Oculex Pharmaceuticals, Inc.), placed in the vitreous base, is effective in treating macular edema associated, among others, with post-cataract surgery (Rana and Pearson, 2006). There are risks, however, of subconjunctival or vitreous hemorrhage and increased IOP.

It is our proposal to combine cataract surgery and postoperative treatment in a single procedure. We developed a sustained release biodegradable DDS linked to the artificial intraocular lens (IOL) by its haptic. We used copolymers of poly(lactic acid) and poly(glycolic acid) (Zhou et al., 1998; Kunou et al., 2000; Yasukawa et al., 2005) and tested DDS in rabbits. The biocompatibility of DDS was analyzed. Insertion of DDS loaded with triamcinolone acetonide (TA) was evaluated with regard to possible reduction of postoperative inflammation.

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-*co*-glycolide) copolymer or PLGA was a mixture 50:50 lactide/glycolide with a molecular weight-average M_w of 34,000 Da (Resomer RG503), 48,000 Da (Resomer RG504) and 80,000 Da (Resomer RG505), abbreviated hereafter as PLGA34000, PLGA48000, and PLGA80000, respectively (Boehringer Ingelheim, Ingelheim, Germany). IOLs (AcrySof® MA 30BA) were supplied by Alcon Pharmaceuticals (Hünenberg, Switzerland). The TA was purchased from Sigma (Buchs, Switzerland); all reagents were of analytical grade.

2.2. Manufacture of drug delivery system

DDS were prepared by dissolving 667 mg of PLGA in 2 g of acetone. For loaded DDS and after complete dissolution of PLGA in acetone, 400 mg TA was homogenously dispersed in a polymer solution by vortexing for 5 min. Unloaded DDS did not contain any active agent. Films were cast by pouring this dispersion into circular Teflon molds (35 mm in diameter) and left to dry at room temperature for 3 days to allow the solvent to evaporate. The films were then separated from the molds and stamped out to obtain discs of around 2 mm in diameter and 1000 μg of TA load. A hole was punched into the center of each disc.

One or two discs were threaded onto an IOL haptic and the resulting combined IOL + DDS system sterilized by ethylene oxide gas.

2.3. Drug concentration in original DDS

DDS discs ($n = 6$) were placed in 25 ml of acetonitrile to dissolve PLGA and TA. The amount of TA was measured by HPLC (Waters LC Module I plus, Milford, MA, USA) using a C-18 reverse-phase column (Nucléosil C-18, 254 mm × 4 mm × 5 μm, Macherey Nagel, Switzerland) at a flow rate of 0.8 ml/min and was detected with a UV spectrophotometer at 236 nm. The mobile phase was composed of a mixture of water (60%) and acetonitrile (40%). Analysis was performed at 20 °C. Under these experimental conditions, retention time of TA was 9.8 min (Felt-Baeyens et al., 2006).

2.4. In vitro release study

The DDS discs ($n = 6$) were incubated in 5 ml of phosphate-buffered solution (0.1 M, pH 7.4) in a closed vial under smooth shaking at 37 °C. At a predetermined interval the entire buffer volume was tested and 5 ml of fresh medium added to the sample vial. After filtration (Durapore, 0.2 μm, Milllipore Switzerland), the amount of TA released into the medium was measured by HPLC and was expressed as a percentage of the initial TA loading in the disc.

2.5. Cataract surgery and implantation

The experiments and surgery on pigmented rabbits were performed in accordance with the Swiss regulations for animal experimentation. Anesthesia was provided by an i.m. injection of 1 ml/kg body weight of a mix (1:3) Rompun® 2% (Bayer, Lyssach, Switzerland): Ketalar® 50 mg/ml (Pfizer, Parke-Davis, Zürich, Switzerland). Mydriasis was induced by one drop of Tropicamide 0.5% SDU Faure (CIBA Vision, Hettlingen, Switzerland), Atropine 1% SDU Faure (Novartis Ophthalmics, Hettlingen, Switzerland) and Néosynéphrine 5% Faure (Europhta, Monaco). Both eyes were kept under slit lamp observation. Lens extraction was performed on the right eye of the pigmented rabbits, the fellow eye being the control. After disinfection with aqueous Betadine® (Mundipharma, Hamilton, Bermuda), Oxybuprocaine 0.4% SDU Faure (Novartis) was instilled directly on the eye to obtain topical anesthesia. During surgery the eye was constantly moistened with drops of Balanced Salt Solution (BSS®, Alcon Laboratories, Inc., Fort Worth, TX, USA). Phacoemulsification was performed with the Series 20000™ Legacy® (Alcon Surgical, Fort Worth, TX, USA) and the pulverized lens aspirated. The IOL or IOL + DDS

system was then inserted. The wounds were sutured with Dafilon 10/0 (B/Braub, Aesculap AG, Tuttlingen, Germany). Topical lomefloxacine (Okacin®, Novartis, Switzerland) was instilled in the eye at the end of surgery and the 3 following days.

The rabbits received the following systems: (1) IOL without DDS (control group, $n = 4$), (2) IOL carrying one unloaded disc of PLGA48000 which did not contain any active agent ($n = 4$), (3) IOL carrying one PLGA48000 disc loaded with TA ($n = 7$), (4) IOL carrying two PLGA48000 discs loaded with TA ($n = 2$), (5) IOL carrying two PLGA34000 discs ($n = 2$), (6) IOL carrying two discs loaded with TA, one PLGA48000 and one PLGA80000 ($n = 2$).

2.6. Evaluation of inflammation

Following implantation the rabbits were clinically examined on days 1, 2, 7, 14, 21 and 42. Corneal edema, conjunctival chemosis, conjunctival hyperemia and secretion were scored according to a semi-quantitative scale from 0 to 3. The clinical score was the sum of the 4 score parameters. The value 0 represented no symptoms/signs and 12 was the maximum. Intraocular pressure (IOP) was measured on days 0, 7, 21 and 42 with a Tono-Pen®XL Applanation Tonometer (Medtronic Ophthalmics).

Aqueous humor (AH) puncture was performed under both systemic and topical anesthesia in both eyes on days 7, 14, 21, 42, 63 and 84. Duplicate samples of AH were dried on slides for inflammatory cell counting after trypan blue staining. Remaining AHs were spun and acellular AH frozen down. Protein concentration was measured with the Coomassie® Plus Protein Assay Reagent Kit (Pierce, Rockford, IL, USA) (Bradford, 1976). Results are given in means ± S.E.M. Data were statistically compared with the Mann–Whitney–Wilcoxon test.

At the end of the experiment, systemic anesthesia, clinical evaluation, IOP measurement and final AH punctures were performed. The rabbits were then euthanized by i.v. injection of pentobarbital. Both eyes were dissected into: conjunctiva, cornea, iris, lens (left eye) or IOL/IOL + DDS (right eye), vitreous humor, retina, choroid, sclera. Blood was drawn for serum preparation. All tissues were frozen and kept for further analysis.

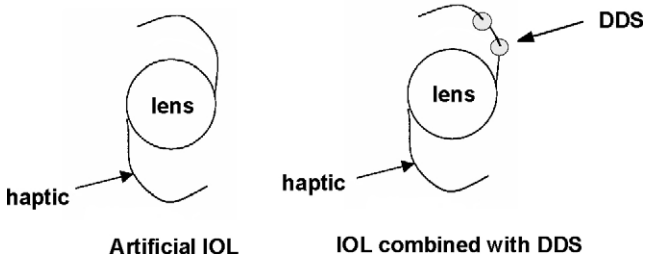

Fig. 1. Schematic representation; artificial IOL as implanted during cataract surgery and IOL combined with two drug delivery system, threaded onto an IOL haptic.

2.7. TA concentration in explanted DDS and ocular tissues

DDS discs that had been placed for various periods of time in the rabbit eye were recovered at euthanasia, detached from the IOL and dissolved in 25 ml of acetonitrile. The amount of TA remaining in the implants was determined by HPLC. The in vivo release of TA was calculated: (amount of TA in the DDS before implantation) − (remaining amount of TA in the recovered DDS)/(amount of TA in the DDS before implantation) × 100. Concentration of TA in ocular tissues was measured according to the described method (Felt-Baeyens et al., 2006).

3. Results

3.1. Characterization of the DDS

A total of seven films were prepared to obtain the DDS discs. All films appeared quite elastic and flexible and the discs were easily shaped from the initial films. Table 1 summarizes the results of disc weight, thickness, diameter and TA content. The loading capacity was approximately 338 ± 7 μg of TA per milligram of polymer. The mean weight of all discs was 2.86 mg and the mean amount of TA per disc was 1050 μg, corresponding to a constant TA loading of 35%. Discs without TA (batch PLGA48000d) were prepared to test the tolerability of the polymeric implant in rabbit eyes.

One or two discs were threaded on an IOL haptic (Fig. 1), leaving freedom of movement between the disc and the haptic. A picture of the IOL combined with two DDS is shown in Fig. 2.

Table 1
Characterization of the drug delivery system

Polymer	Batch	Average weight (mg)[a]	Average thickness (mm)[a]	Average diameter (mm)[a]	Average amount of TA per DDS (μg)[a]
PLGA34000	PLGA34000a	2.90 ± 0.06	0.3	2.1	1040 ± 4
	PLGA34000b	2.97 ± 0.11	0.3	2.0	1042 ± 61
PLGA48000	PLGA48000a	2.94 ± 0.11	0.3	2.1	1021 ± 47
	PLGA48000b	2.96 ± 0.04	0.3	2.0	1003 ± 19
	PLGA48000c	3.12 ± 0.06	0.3	2.1	1158 ± 23
	PLGA48000d[b]	2.16 ± 0.06	0.2	2.0	–
PLGA80000	PLGA80000	2.97 ± 0.16	0.3	2.1	1038 ± 22

[a] Mean of six measurements.
[b] DDS without TA.

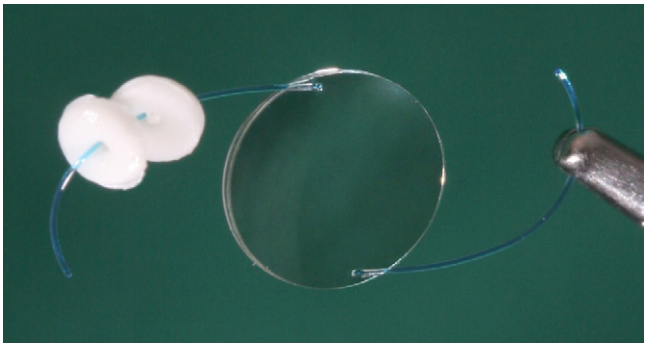

Fig. 2. Picture of the IOL combined with two DDS.

3.2. In vitro release of TA

The cumulative release of TA from the different types of disc is plotted in Fig. 3. Each vial contained one DDS disc (PLGA34000a, PLGA48000a and PLGA80000). The three types of device show different TA release profiles due to differences in the M_w of the polymer. PLGA480000a discs showed a tri-phasic release profile with an initial burst, and a second stage followed by a second burst. The initial burst (6%) occurred within 1 week and was observed for the three types of DDS, with different release percentages. For the PLGA48000a disc, 28 g of TA was released during the first 24 h. After this initial burst, TA was released slowly over 3 weeks (diffusion phase). The second burst occurred 4 weeks after incubation. For implants PLGA34000a and PLGA80000, no second burst was observed.

Although PLGA34000a discs showed a more rapid release than PLGA48000a discs at the beginning of the incubation, the total amount of drug released was almost the same for both discs at the end of the in vitro assay: 282 μg of TA (29%) was released from PLGA34000a discs after 73 days and 285 μg of TA (24%) from PLGA48000a implants after 71 days. The in vitro studies were terminated at days 71 or 73 when some of the DDS from the two batches were partly broken up or dissolved.

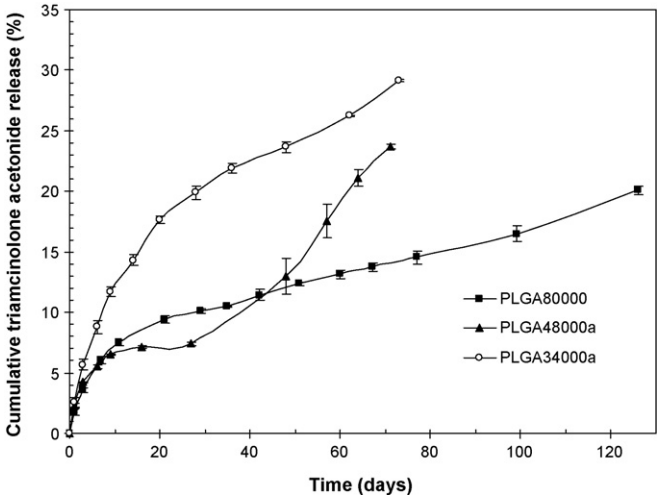

Fig. 3. Effects of the M_w of PLGA on in vitro release from DDS at constant TA loading of 35%. (◯) DDS PLGA34000a, (▲) DDS PLGA48000a, (■) DDS PLGA80000. The values are means ± S.D. ($n = 6$).

The DDS from batch PLGA80000 showed rapid release with an initial burst (Fig. 3) without the latency period that was observed with the PLGA48000a. TA release was gradual and very slow. After 18 weeks (day 126) of in vitro investigations, 20% of TA was released from the PLGA80000 implants, corresponding to 216 μg of TA.

The in vitro release profile per disc obtained with DDS PLGA48000 remained the same whether one or two discs were implanted. After 50 days, one DDS released 156 μg of TA while two DDS discs released 330 μg. Thus two discs delivered twice as much TA as one disc. This demonstrates that the medium of the vial did not reach saturation in TA concentration at 37 °C.

Altogether these results confirmed that a decrease in the M_w of PLGA results in a higher rate of drug release.

3.3. Effect of DDS on inflammation

Lens extraction and IOL insertion induced inflammation. At day 7 after surgery, more than 10 mg/ml protein was measured in AH (Fig. 4, IOL) and around 20 cells/μl AH were counted in AH (Fig. 5, IOL). Both parameters progressively returned to normal values within around 4 weeks. The clinical score reached a value of 4–6, at day 7 after the surgery. The value then progressively decreased with a time interval of 4 weeks (not shown).

In the contralateral control eyes, protein concentration was null at any time point and the number of inflammatory cells were <2 (not shown). These values confirm that control eyes are in a calm situation and do not show any inflammation.

Unloaded 48000 DDS inserted with the IOL did not induce greater inflammation than the IOL without DDS. Values for protein concentrations and number of inflammatory cells within the AH were comparable to those obtained after simple cataract surgery (Figs. 4 and 5, IOL + unloaded 48000). Insertion of the unloaded DDS did not worsen the clinical score either (not

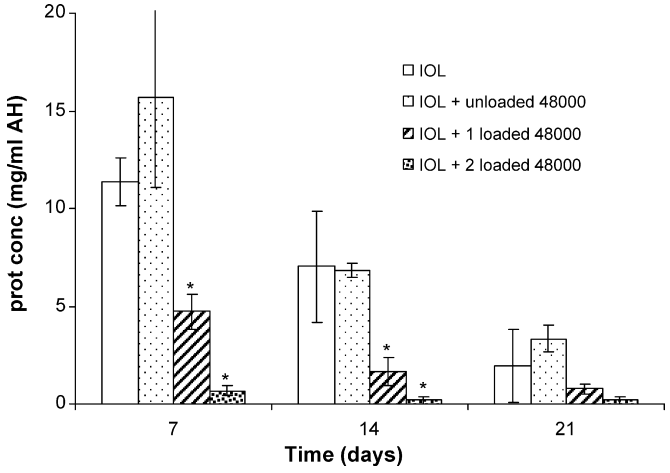

Fig. 4. Inflammation after cataract surgery, as measured by protein concentration within the aqueous humor in mg/ml. Following devices were inserted: IOL (control), IOL + unloaded DDS PLGA48000, IOL + one loaded DDS PLGA48000, IOL + two loaded DDS PLGA48000. *Results are significantly different from IOL control. At 7 days, for IOL + one loaded 48000, $P = 0.0084$; for IOL + two loaded 48000, $P = 0.0133$. At 14 days, for IOL + one loaded 48000, $P = 0.0324$; for IOL + two loaded 48000, $P = 0.0502$.

shown). These results prove that PLGA and DDS conformation are biocompatible and well tolerated in the rabbit eye.

One 48000 DDS loaded with TA significantly reduced protein concentration within the AH compared to an IOL without DDS at day 7 ($P = 0.0084$) and day 14 ($P = 0.0324$) (Fig. 4, IOL + one loaded 48000). The insertion of two loaded 48000 DDS reduced inflammatory protein exudation within the AH to near untreated levels (Fig. 4, IOL + two loaded 48000). In comparison to IOL control, the significant P values were 0.0133 and 0.0502 for day 7 and 14, respectively. The number of cells in the AH was also reduced (Fig. 5, IOL + two loaded 48000). At day 14, the result obtained with two loaded 48000 DDS was significantly different from the control ($P = 0.0023$). Insertion of two loaded 48000 DDS considerably reduced the clinical score that returned to null at day 14 after surgery (not shown). These observations prove that TA is delivered from the DDS within the first days after surgery and has a therapeutic effect during at least 21 days afterwards. Two discs are more efficient than one in reducing postoperative inflammation.

In all the experiments, the non-implanted fellow (left) eyes showed no protein exudation within the AH at any time, whatever the type of DDS implanted in the right eye. The clinical score of the non-implanted eyes also stayed at zero (not shown).

Mean values of intraocular pressure (IOP) varied between 7 and 15 mmHg during the time of the experiments, with no significant difference between the control eyes and those undergoing cataract surgeries. The DDS loaded with TA did not induce pathological changes in IOP (not shown).

Experiments were also conducted over longer periods of time (days 42, 63 and 84) and were compared to lens extraction and IOL insertion with no DDS. Dual DDS showing a progressive continuous in vitro long-term delivery without a second burst (not shown) were chosen for this comparison. The device comprising of one 48000 DDS + one 80000 DDS was more effective in inhibiting inflammation than that of two 34000 DDS. At day 63, the 48000 + 80000 device significantly reduced protein concentration in the AH ($P = 0.0119$) (Fig. 6) and the number of inflammatory cells ($P = 0.0423$) (Fig. 7). At day 84, there was a tendency toward a significant inhibition of the number of cells in AH ($P = 0.0747$).

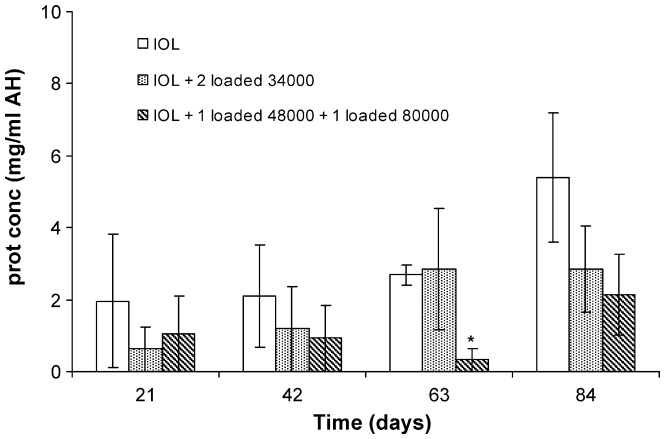

Fig. 6. Long-term effect of two DDS as measured by the protein concentration within the aqueous humor. Insertion of either IOL (control), IOL + two loaded DDS PLGA34000 or IOL + one DDS PLGA48000 + one DDS PLGA80000. At day 63, one loaded 48000 + one loaded 80000 significantly reduced inflammation, $P = 0.0119$.

3.4. In vivo release of TA

The profile of in vitro (PLGA48000a) and in vivo (PLGA48000b) cumulative TA release is compared in Fig. 8. In contrast with the in vitro release, no initial burst was observed in vivo. After 1 week, the in vivo discs released as little as 11 μg (=0.6%) of TA, whereas the in vitro implants released up to 66 μg (=6%). At day 20, approximately the same amount was released from the in vitro and in vivo discs (around 90 μg TA, corresponding to 7.5%). Thereafter, DDS released the drug faster in vivo than it did in vitro. After day 42, the mean amount released in vivo and in vitro was 410 μg (40.5%) and 156 μg (13%), respectively. DDS recovered from rabbit eyes were intact at day 21 but distorted at day 42.

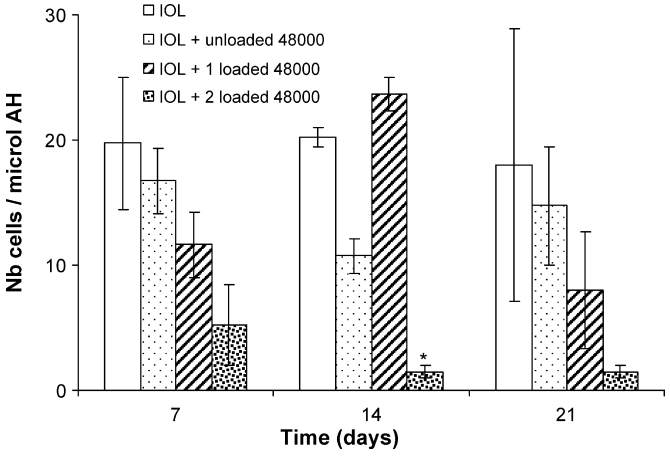

Fig. 5. Inflammation after cataract surgery, as measured by the number of inflammatory cells per microliter of aqueous humor. See Fig. 4. *Results are significantly different from IOL control at 14 days, for IOL + two loaded 48000, $P = 0.0023$.

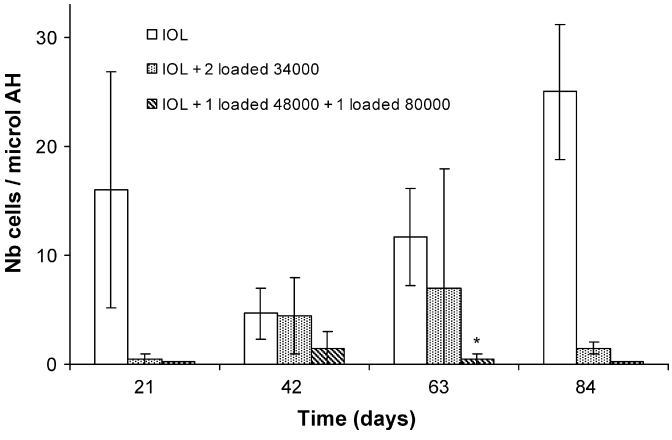

Fig. 7. Long-term effect of two DDS as measured by the number of inflammatory cells within the aqueous humor. See Fig. 6. At day 63, one loaded 48000 + one loaded 80000 significantly reduced inflammation, $P = 0.0423$. At day 84, one loaded 48000 + one loaded 80000 had the tendency to reduce inflammation, $P = 0.0747$.

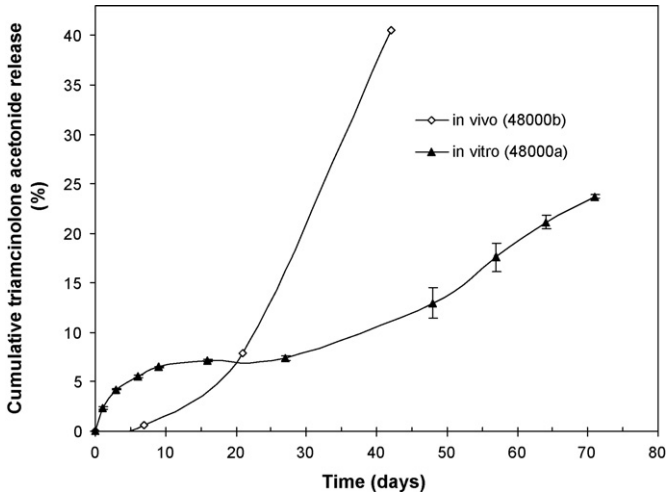

Fig. 8. In vivo and in vitro release profile of TA from the DDS prepared with PLGA48000. (▲) In vitro DDS (PLGA48000a), (◊) in vivo DDS (PLGA48000b). The data are mean percentages of the initial concentration within the DDS ($n = 6$ in vitro and $n = 2$ in vivo).

To increase the amount of TA potentially delivered in the eye, two discs (PLGA48000b) were implanted in rabbit eyes (rabbits 43 and 44) (Table 2). In this dual system, a mean of 39% of the initial amount of TA was released after 6 weeks from the two discs, corresponding to 761 μg of TA.

The same investigation was carried out with the PLGA 34000b batch using two DDS per IOL. This situation also demonstrated that the total amount of TA released after 12 weeks was higher in vivo than in vitro. An average of 59% of the initial amount of TA was released in vivo, corresponding to 840 μg of TA (Table 2), whereas only 29% was released in vitro after 73 days (10.5 weeks) (Fig. 3). Altogether, these results confirmed that the drug delivery system could release a higher amount of TA in vivo than in vitro.

The combination of PLGA48000 and PLGA80000 on one IOL (one PLGA48000c + one PLGA80000) was also tested in vivo. Unfortunately, the total amount of TA released could not be determined as during removal of the DDS from the capsular bag of the lens at the end of the in vivo investigation (12 weeks), the discs immediately disintegrated, making any accurate measurement of recovered TA impossible.

Table 2
Total amount of TA released from the DDS during the in vivo investigation

Rabbit #	Batch	TA released		Time (week)
		%	(μg)	
40	PLGA48000b	1.2	12	1
39		0	0	
42		7.4	74	3
37		8.1	80	
38		8.0	81	
45		55	564	6
46		26	256	
43[a]		31	608	
44[a]		46	914	
51[a]	PLGA34000b	61	779	12
52[a]		56	901	

[a] Rabbits implanted with IOL + two loaded DDS.

4. Discussion

We have shown that a biodegradable DDS composed of PLGA matrix provides an effective method for intraocular TA delivery following cataract surgery. Our "all in one" system containing both DDS and IOL has a double advantage. First of all, the DDS is evaluated in a model of inflammation (cataract surgery), using inflammation parameters within the anterior chamber. Secondly, patients undergoing cataract extraction often suffer from other diseases (uveitis, various retinopathies, AMD) that may be worsened by surgical intervention and require additional treatments (El Harazi and Feldman, 2001; Taylor and Keeffe, 2001). Insertion of a linked DDS during cataract surgery would alleviate postoperative treatments and minimize the relapse of pre-existing pathologies.

In vitro, TA release profile from the DDS was controlled by varying the M_w of PLGA (Yasukawa et al., 2005). Our discs, prepared with PLGA48000a, showed a tri-phasic release profile (Tojo and Isowaki, 2001). The initial burst, lasting 1 week, may result from the rapid release of the drug absorbed on the surface of the disc. During the second stage, TA was released slowly over 3 weeks (diffusion phase). Swelling and disintegration of the polymer matrix were responsible for the second burst and occurred 4 weeks after incubation. For PLGA80000 discs, no second burst was observed within 18 weeks, suggesting that the inner polymer degrades very little. With PLGA34000a discs, no second burst was observed. This may be due to the faster degradation rate of PLGA34000a that allowed water to transit via channels between the surface and the inside of the implant (Hashizoe et al., 1994). Thus, TA can be released continuously and at a relatively high rate by diffusion throughout the water channels.

In vivo, TA release rate is higher than in vitro, probably due to both increased TA solubility and increased degradation of the polymer in vivo (Okabe et al., 2003). A saturation of the solution in vitro could be excluded, since two DDS in the same vial showed the same in vitro release profile as one DDS. At the early stage in vivo, water absorption of the implant in the eye may be less than in vitro, implying that the water channels could not develop in the matrix and the drug could not be delivered. Later, the drug may be released after communication is established between the inner and outer layers.

We demonstrated that our biodegradable unloaded DDS made of PLGA is biocompatible as it causes no greater inflammation than lens extraction and implantation of an IOL without DDS. Such DDS has been shown to be well tolerated (Theng et al., 2003; Sakurai et al., 2003). Our study shows that DDS loaded with around 1 mg of TA significantly reduced inflammation in the first days following surgery. Two DDS with a total load of 2 mg TA were more efficient than one DDS.

Increase of IOP is one of the usual side-effects of TA, after intraocular injections (Ciulla et al., 2004; Jonas et al., 2005a). In our study, the absence of increased IOP is probably due to the very low steroid concentrations in the eye. The system of

two PLGA48000 discs initially contained 2 mg of TA. Because of slow delivery and turn-over of TA, we were able to ascertain that less than 150 μg is present in the anterior chamber.

Complications in eyes following surgery are currently treated by intravitreal injections of TA which reduce proliferative vitreoretinopathy and treat macular edema (Antcliff et al., 2001; Audren et al., 2004; Yasukawa et al., 2005; Jonas et al., 2005b). However, they need to be repeated and may lead to hemorrhage, retinal detachment or infections (Jonas et al., 2005b). Such treatments could be replaced by combined DDS and IOL.

We were able to inhibit postoperative inflammation with two 48000 DDS at short term (day 21). For the longer term (days 63, 84), a system comprised of one 48000 disc and one 80000 disc was more effective in reducing inflammation. The use of the 48000 disc provides drug release during the first weeks and the second, 80000 disc, allows release for a longer period of time (Yasukawa et al., 2005).

Another type of DDS associated with IOL has been tested in rabbits (Siqueira et al., 2006). The IOL, made of poly(methyl methacrylate), was modified by adding two rings of 1 mm diameter. DDS, made of PLGA, had a weight of 1.5 mg and contained 25% dexamethasone (DX). Two DDS were attached to each lens ring. This system was shown to deliver therapeutic concentrations of DX during 9 days in both aqueous and vitreous humors.

The biodegradable DDS associated with IOL shows many advantages over conventional eye treatments (Yasukawa et al., 2005). Lower drug concentrations are required to produce identical effects and side effects are reduced (Tan et al., 1999). Patient non-compliance is eliminated. Biodegradable DDS makes a second surgery to remove the implant unnecessary.

DDS combined with IOL has a real advantage over other systems, since the implant is immobilized within the capsule. PLGA rods loaded with DX (Surodex®, Oculex Pharmaceuticals, Inc.), freely inserted in the anterior or posterior chamber of patients during cataract surgery, were safe and efficient in reducing inflammatory symptoms (Tan et al., 1999; Chang et al., 1999; Tan et al., 2001; Lee et al., 2003; Wadood et al., 2004). However, a significant implant migration to the anterior chamber was noted, affecting visual acuity (Tan et al., 2001). Implant residues were still present after one year and the long-term ocular discomfort was not negligible for the patients. Finally, focal peripheral anterior synechiae were observed in 22 out of 71 eyes (Tan et al., 2001).

Our DDS could be adapted by incorporation of other steroids to treat severe uveitis (Okabe et al., 2003; Kato et al., 2004; Cahill and Jaffe, 2006), ganciclovir to treat cytomegalovirus (CMV) retinitis (Kunou et al., 2000; Yasukawa et al., 2005), or antibiotics to treat various types of endophthalmitis. The DDS could also treat proliferative vitreoretinopathy, choroidal neovascularization and diabetic retinopathy (Zhou et al., 1998; Yasukawa et al., 2002). One of the most appropriate applications would be the treatment of AMD. To date, AMD represents the most common source of blindness in elderly people in developed countries. Among these patients, 15% present the wet form, leading to choroidal neovascularization (Ciulla et al., 2004; Yasukawa et al., 2005). Anti-angiogenic agents like anti-VEGF have been demonstrated to greatly inhibit neovascularization (Ambati et al., 2003). The difficulty is to maintain effective concentrations of this active agent. The implantation of a DDS containing an anti-VEGF, or antisense oligonucleotides, could be an innovative treatment.

In conclusion, our results suggest that a new intraocular DDS using a biodegradable polymer device placed during cataract surgery may be useful to decrease postoperative inflammation and reduce the development of postoperative complications. The major advantage of the intraocular DDS is the possibility to combine cataract surgery and postoperative treatment in a single procedure.

Acknowledgements

Our warm thanks go to Catherine Guex-Crosier and Dr. Hana Abou Zeid for their help in surgery, to the photographers M. Curchod, S. Fromhold and Y. Leuba and to the animal keeper N. Terrier. This work has been supported by the Swiss National Foundation (#3200B0-105337/1) and the Novartis Consumer Health Foundation.

References

Adamis, A.P., Shima, D.T., 2005. The role of vascular endothelial growth factor in ocular health and disease. Retina 25, 111–118.

Ambati, J., Ambati, B.K., Yoo, S.H., Ianchulev, S., Adamis, A.P., 2003. Age-related macular degeneration: etiology, pathogenesis, and therapeutic strategies. Surv. Ophthalmol. 48, 257–293.

Antcliff, R.J., Spalton, D.J., Stanford, M.R., Graham, E.M., Ffytche, T.J., Marshall, J., 2001. Intravitreal triamcinolone for uveitic cystoid macular edema: an optical coherence tomography study. Ophthalmology 108, 765–772.

Audren, F., Tod, M., Massin, P., Benosman, R., Haouchine, B., Erginay, A., Caulin, C., Gaudric, A., Bergmann, J.F., 2004. Pharmacokinetic-pharmacodynamic modeling of the effect of triamcinolone acetonide on central macular thickness in patients with diabetic macular edema. Invest. Ophthalmol. Vis. Sci. 45, 3435–3441.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Cahill, M.T., Jaffe, G.J., 2006. Intraocular sustained-release drug delivery in uveitis. In: Jaffe, G.J., Ashton, P., Pearson, P.A. (Eds.), Intraocular Drug Delivery. Taylor & Francis Group, New York, pp. 265–278.

Chang, D.F., Garcia, I.H., Hunkeler, J.D., Minas, T., 1999. Phase II results of an intraocular steroid delivery system for cataract surgery. Ophthalmology 106, 1172–1177.

Ciulla, T.A., Walker, J.D., Fong, D.S., Criswell, M.H., 2004. Corticosteroids in posterior segment disease: an update on new delivery systems and new indications. Curr. Opin. Ophthalmol. 15, 211–220.

El Harazi, S.M., Feldman, R.M., 2001. Control of intra-ocular inflammation associated with cataract surgery. Curr. Opin. Ophthalmol. 12, 4–8.

Eyetech Study Group, 2003. Anti-vascular endothelial growth factor therapy for subfoveal choroidal neovascularization secondary to age-related macular degeneration: phase II study results. Ophthalmology 110, 979–986.

Felt-Baeyens, O., Eperon, S., Mora, P., Limal, D., Sagodira, S., Breton, P., Simonazzi, B., Bossy-Nobs, L., Guex-Crosier, Y., Gurny, R., 2006. Biodegradable scleral implants as new triamcinolone acetonide delivery systems. Int. J. Pharm. 322, 6–12.

Hankinson, S.-E., 2000. Epidemiology of age-related cataract. In: Albert, D.M., Jakobiec, F.A., Azar, D.T., Gragoudas, E.S. (Eds.), Principles and Practice of Ophthalmology, vol. 1. W.B. Saunders Company, Philadelphia, pp. 511–521.

Hashizoe, M., Ogura, Y., Kimura, H., Moritera, T., Honda, Y., Kyo, M., Hyon, S.-H., Ikada, Y., 1994. Scleral plug of biodegradable polymers for controlled drug release in the vitreous. Arch. Ophthalmol. 112, 1380–1384.

Jonas, J.B., Degenring, R.F., Kreissig, I., Akkoyun, I., Kamppeter, B.A., 2005a. Intraocular pressure elevation after intravitreal triamcinolone acetonide injection. Ophthalmology 112, 593–598.

Jonas, J.B., Kreissig, I., Degenring, R., 2005b. Intravitreal triamcinolone acetonide for treatment of intraocular proliferative, exudative, and neovascular diseases. Prog. Retin. Eye Res. 24, 587–611.

Kato, A., Kimura, H., Okabe, K., Okabe, J., Kunou, N., Ogura, Y., 2004. Feasibility of drug delivery to the posterior pole of the rabbit eye with an episcleral implant. Invest. Ophthalmol. Vis. Sci. 45, 238–244.

Kunou, N., Ogura, Y., Yasukawa, T., Kimura, H., Miyamoto, H., Honda, Y., Ikada, Y., 2000. Long-term sustained release of ganciclovir from biodegradable scleral implant for the treatment of cytomegalovirus retinitis. J. Control. Release 68, 263–271.

Lee, S.Y., Chee, S.P., Balakrishnan, V., Farzavandi, S., Tan, D.T., 2003. Surodex in paediatric cataract surgery. Br. J. Ophthalmol. 87, 1424–1426.

McGhee, C.N., Dean, S., Danesh-Meyer, H., 2002. Locally administered ocular corticosteroids: benefits and risks. Drug Saf. 25, 33–55.

Miyake, K., Masuda, K., Shirato, S., Oshika, T., Eguchi, K., Hoshi, H., Majima, Y., Kimura, W., Hayashi, F., 2000. Comparison of diclofenac and fluorometholone in preventing cystoid macular edema after small incision cataract surgery: a multicentered prospective trial. Jpn. J. Ophthalmol. 44, 58–67.

Okabe, J., Kimura, H., Kunou, N., Okabe, K., Kato, A., Ogura, Y., 2003. Biodegradable intrascleral implant for sustained intraocular delivery of betamethasone phosphate. Invest. Ophthalmol. Vis. Sci. 44, 740–744.

Parke, D.W., 2003. Intravitreal triamcinolone and endophthalmitis. Am. J. Ophthalmol. 136, 918–919.

Rana, Z.A., Pearson, P.A., 2006. Pharmacologic treatment in diabetic macular edema. In: Jaffe, G.J., Ashton, P., Pearson, P.A. (Eds.), Intraocular Drug Delivery. Taylor & Francis Group, New York, pp. 291–300.

Sakurai, E., Nozaki, M., Okabe, K., Kunou, N., Kimura, H., Ogura, Y., 2003. Scleral plug of biodegradable polymers containing tacrolimus (FK506) for experimental uveitis. Invest. Ophthalmol. Vis. Sci. 44, 4845–4852.

Simone, J.N., Whitacre, M.M., 2001. Effects of anti-inflammatory drugs following cataract extraction. Curr. Opin. Ophthalmol. 12, 63–67.

Siqueira, R.C., Filho, E.R., Fialho, S.L., Lucena, L.R., Filho, A.M., Haddad, A., Jorge, R., Scott, I.U., Cunha, A.S., 2006. Pharmacokinetic and toxicity investigations of a new intraocular lens with a dexamethasone drug delivery system: a pilot study. Ophthalmologica 220, 338–342.

Tan, D.T., Chee, S.P., Lim, L., Lim, A.S., 1999. Randomized clinical trial of a new dexamethasone delivery system (Surodex) for treatment of post-cataract surgery inflammation. Ophthalmology 106, 223–231.

Tan, D.T., Chee, S.P., Lim, L., Theng, J., Van Ede, M., 2001. Randomized clinical trial of Surodex steroid drug delivery system for cataract surgery: anterior versus posterior placement of two Surodex in the eye. Ophthalmology 108, 2172–2181.

Taylor, H.R., Keeffe, J.E., 2001. World blindness: a 21st century perspective. Br. J. Ophthalmol. 85, 261–266.

Theng, J.T., Ti, S.E., Zhou, L., Lam, K.W., Chee, S.P., Tan, D., 2003. Pharmacokinetic and toxicity study of an intraocular cyclosporine DDS in the anterior segment of rabbit eyes. Invest. Ophthalmol. Vis. Sci. 44, 4895–4899.

Tojo, K., Isowaki, A., 2001. Pharmacokinetic model for in vivo/in vitro correlation of intravitreal drug delivery. Adv. Drug Deliv. Rev. 52, 17–24.

Wadood, A.C., Armbrecht, A.M., Aspinall, P.A., Dhillon, B., 2004. Safety and efficacy of a dexamethasone anterior segment drug delivery system in patients after phacoemulsification. J. Cataract Refract. Surg. 30, 761–768.

Wang, J.J., Klein, R., Smith, W., Klein, B.E., Tomany, S., Mitchell, P., 2003. Cataract surgery and the 5-year incidence of late-stage age-related maculopathy: pooled findings from the Beaver Dam and Blue Mountains eye studies. Ophthalmology 110, 1960–1967.

Wright, P.L., Wilkinson, C.P., Balyeat, H.D., Popham, J., Reinke, M., 1988. Angiographic cystoid macular edema after posterior chamber lens implantation. Arch. Ophthalmol. 106, 740–744.

Yasukawa, T., Kimura, H., Tabata, Y., Miyamoto, H., Honda, Y., Ogura, Y., 2002. Sustained release of cis-hydroxyproline in the treatment of experimental proliferative vitreoretinopathy in rabbits. Graefes Arch. Clin. Exp. Ophthalmol. 240, 672–678.

Yasukawa, T., Ogura, Y., Sakurai, E., Tabata, Y., Kimura, H., 2005. Intraocular sustained drug delivery using implantable polymeric devices. Adv. Drug Deliv. Rev. 57, 2033–2046.

Zhou, T., Lewis, H., Foster, R.E., Schwendeman, S.P., 1998. Development of a multiple-drug delivery implant for intraocular management of proliferative vitreoretinopathy. J. Control. Release 55, 281–295.