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Biodegradable scleral implants as new triamcinolone acetonide delivery systems

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Abstract

The goal of this study was to develop ocular scleral implants able to release triamcinolone acetonide (TA) overall several months. Scleral discs were manufactured by a compression-molding method using a new synthetic polymer, poly(methylidene malonate) (PMM2.1.2), as matrix. Implants with good mechanical properties adapted for in vivo implantation have been obtained when using high M_w PMM2.1.2 (100,000–150,000 Da) associated with ethoxylated derivatives of stearic acid (Simulsol®) or oligomers of methylidene malonate as plasticizer. After implantation in rabbit eyes, scleral implants showed a good ocular biocompatibility. Indeed, the clinical follow-up and ocular inflammation parameters, such as inflammatory cell number and protein content in aqueous humor, demonstrated that implants were well tolerated and did not provoke abnormal inflammation. Implants were able to release significant concentrations of TA in the vitreous and the sclera throughout 5 weeks.

Keywords: Scleral implant; Biodegradable polymer; Biocompatibility; Triamcinolone acetonide

1. Introduction

Treatment of severe ocular pathologies such as uveitis, proliferative vitreoretinopathy and retinitis remains a major challenge in ophthalmology. In fact, treatment of such vitreoretinal diseases is based on conventional pathways of drug administration, e.g., topical instillations, subconjunctival injections and systemic administration, which achieve low levels of drugs at the target site. All of these routes present anatomical as well as physiological barriers. Topical instillations hugely limits penetration of drugs into the vitreous due to the poor permeability of the cornea and efficient protective mechanisms such as solution drainage, lachrymation and diversion of exogenous substances into the systemic circulation via conjunctival absorption (Lee et al., 1989). Subconjunctival injections give uncertain and, most often, inadequate concentrations of drugs into the vitreous (Kunou et al., 2000). Finally, systemic treatment needs the administration of large amounts of drug, leading to both poor intravitreal drug concentration mainly due to both blood–aqueous and blood–retinal barriers on one side and important side effects on the other side.

Intravitreal injection of 0.1 ml of the marketed suspension Kenacort® (Bristol-Myers Squibb AG, Baar, Switzerland) containing 4 mg of triamcinolone acetonide (TA) is beginning a more accepted mean of vitreous drug delivery being recognized to achieve more effective drug concentrations. Such intravitreal injections replace posterior subtenon injection of 1 ml of Kenacort® containing 40 mg of TA, that sometimes lead to severe ocular hypertension (McGhee et al., 2002). However, intravitreal injections need to be repeated often frequently which may cause severe complications including vitreous hemorrhage, retinal detachment or infections (Kimura et al., 1994).

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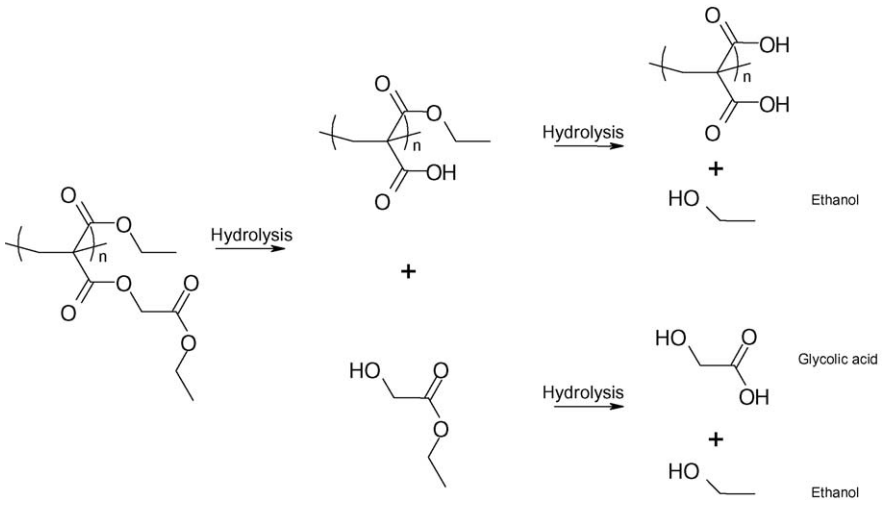

Fig. 1. Chemical structure and degradation pathways of PMM2.1.2 polymers (Roy et al., 1997; Lescure et al., 1994).

A sustained-release drug delivery system such as biodegradable implants offering minimal side effects and invasion may be an interesting means to release drugs directly into the vitreous and ensuring therapeutic concentrations over a prolonged period of time. It has been assumed that transscleral delivery may be an effective method of achieving therapeutic concentrations of drugs in the posterior segment of the eye (Ambati and Adamis, 2002; Geroski and Edelhauser, 2001).

Studies in this field led to the development of two different types of systems, namely non-biodegradable and biodegradable implants. On one hand non-biodegradable implants based on ethylene vinyl acetate (EVA) and/or polyvinylalcohol have been demonstrated to be successful delivery systems for prolonged release of drugs such as ganciclovir (Sanborn et al., 1992; Anand et al., 1993; Martin et al., 1994), cyclosporine A on a higher number of animals (Pearson et al., 1996), dexamethasone (Cheng et al., 1995; Hainsworth et al., 1996) and more recently, fluocinolone acetonide (Jaffe et al., 2000). On the other hand, major disadvantage of non-biodegradable systems is the need of a second surgery to remove the system and replace it to prevent any risk of fibrous encapsulation. In this way, biodegradable systems have gained interest to eliminate the removal step. Various biodegradable polymers have been used to design such biodegradable solid dosage forms (cylinders, nail-like scleral plugs, discs, rods and tablets), main works being dedicated to the use of poly(lactic acid) and/or poly(glycolic acid) and their copolymers (Miyamoto et al., 1997; Zhou et al., 1998; Kunou et al., 2000; Yasukawa et al., 2001).

The aim of the present project was to develop a poly(methylidene malonate) (PMM2.1.2) based scleral implant able to achieve a prolonged release over several months of the anti-inflammatory drug TA to avoid repeated injections associated with the Kenacort® treatment. PMM2.1.2 is a synthetic polymer that has been mainly used for the manufacture of particulates systems (Breton et al., 1998; Roy et al., 1997; Chan et al., 2004). Its use for the development of intraocular implants seems of peculiar interest since it has been demonstrated to be non-toxic and biodegradable leading to the formation of non-toxic products including ethanol and glycolic acid (Breton et al., 1994; Lescure et al., 1994).

Implants manufactured by a compression-molding method have been evaluated regarding their mechanical properties, drug content and stability to gamma-sterilization. In vivo studies were directed toward the ocular biocompatibility of PMM2.1.2 implants. Preliminary results of TA release in the vitreous are presented.

2. Materials and methods

2.1. Materials

PMM2.1.2 (Fig. 1) is a biodegradable polymer that was supplied by Virsol (Paris, France). Several types of polymer have been tested, M_w ranging from about 10,000 to 250,000 Da as specified by the supplier.

TA was purchased from Sigma (Buchs, Switzerland). Five plasticizers have been used at several concentrations as described in Table 1.

Table 1
Types and concentration of plasticizers used for scleral implants manufacture

Plasticizer	Concentration (%, w/w)
PEG[a] 200	20
	40
PEG[a] 400	20
	40
Simulsol[b]	10
Myvacet[c]	5
	10
OMM2.1.2[d]	5
	7.5
	10

[a] Polyethyleneglycols.
[b] Ethoxylated derivatives of stearic acid (Simulsol®, Seppic, France).
[c] Monoglycerides derivatives (Myvacet®, Eastman).
[d] Methylidene malonate 2.1.2 oligomers (OMM2.1.2).

2.2. Manufacture and characterization of scleral implants

Scleral implants based on PMM2.1.2 were manufactured by a modified compression-molding method (Schwach-Abdellaoui et al., 2001). Briefly, the polymer was solubilized in tetrahydrofuran (THF, Romil, Switzerland). After casting on Petri dishes, THF was eliminated under controlled conditions to avoid bubble formation. The films were then compressed (Specac®) at 3000 t during 2 min to obtain a standardized thickness of 1 mm and, finally, implants of 8 mm of diameter were manufactured using a die. Drug loaded implants were manufactured according to the same process, TA being incorporated during the solubilization step of PMM2.1.2 into THF. Implants were mainly characterized regarding their size, mechanical properties and drug content. TA was dosed by high liquid performance chromatography (HPLC) after complete dissolution of the discs in acetonitrile (method description in Section 2.5). Residual THF in scleral implants has been evaluated by equilibrium headspace gas chromatography on a HP5890 gas chromatograph coupled with a FID detector (hydrogen 30 ml/min, air 400 ml/min) for quantitative analysis. The column used was a HP Plot Q (0.53 mm × 40 μm × 15 m). Samples were analyzed in airtight closed 10 ml vials, PMM2.1.2 being dissolved in 5 ml benzylic alcohol as matrix. Samples were equilibrated for 10 min. Briefly, conditions of temperature were the following: injector 220, column 120, detector 260 and samples 160 °C.

Implants were further sterilized by gamma irradiation using a ^{60}Cobalt source at 25 kGy as recommended by the European Pharmacopoeia 4 (Studer AG Werk Hard, Däniken, Switzerland). To prevent deleterious thermal effects, gamma irradiation was carried out at defined temperature using dry ice. Effect of the sterilization process on the polymeric matrix was evaluated by size exclusion chromatography (SEC). SEC was performed with a Waters® 600E instrument equipped with a series of 4 Styragel HR® columns (Waters, Switzerland) and a refractive index detector Waters® 410. Determinations were carried out at 30 °C in THF as eluant (1 ml/min). To calibrate the system, narrow distributed polystyrene standards (PS) have been used in the M_w range from 5900 to 96,400 Da. Hence, PMM2.1.2 M_w was expressed as PS equivalent.

Qualitative and/or quantitative influence of gamma irradiation on TA was assessed by HPLC on scleral implants before and after sterilization after drug extraction in acetonitrile.

2.3. Biocompatibility

The experiments with rabbits were done in accordance with the Swiss regulations for animal experimentation. Rabbits were anaesthetized with 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). The right eye was chosen for implantation/injection and the contralateral eye was used as a control. After disinfections with aqueous Betadine® (Mundipharma, Hamilton, Bermuda), Oxybuprocaine 0.4% SDU Faure (Novartis Ophthalmics, Hettlingen, Switzerland) was instilled into the eye to obtain topical anesthesia. During surgery, the eye was constantly moistured with drops of balanced salt solution (BSS®, Alcon, Fort Worth, TX, USA), a sterile irrigating solution. The conjunctiva was opened in the upper temporal quadrant and the sclera was exposed. The implant was fastened to the sclera by 5-0 Ticron* sutures (Sherwood* Davis & Geck, Adliswil, Switzerland) and the conjunctival wound sutured with 7/0 vicryl® (Johnson & Johnson, Spreitenbach, Switzerland). A topical antibiotic, Spersanicol® containing chloramphenicol (CIBA Vision, Hettlingen, Switzerland) was instilled in the eye at the end of surgery and during the 3 following days.

The biocompatibility of the polymer PMM2.1.2 was assessed by the total clinical score (defined below) and retinal ophthalmoscopy, on a daily basis during the first 4 days following surgery and then once a week until the end of the experiment. Rabbits were scored from 0 to 3 according to (1) conjunctival hyperemia, (2) chemosis and (3) edema. The total clinical score was the sum of the 3 scores, where 0 represented no symptoms/signs and 9 was the maximum. After systemic and topical anesthesia at the end of the experiment, mydriasis was induced by tropicamide 0.5% SDU Faure (CIBA Vision, Hettlingen, Switzerland) and the fundus of the eye was observed to detect retinal lesions. Aqueous humor (AH) was withdrawn from both implanted and contralateral control eyes. Biocompatibility was also assessed by two signs of ocular inflammation in AH, the total number of inflammatory cells and protein concentration, as measured by the Coomassie Plus Protein Assay (Pierce, Rockford, Il, USA) (Bradford, 1976).

Biocompatibility of the polymer was analyzed on 13 rabbits over a period of 56 days. Biocompatibility has been assessed using implants formulated with PMM2.1.2 polymer of $M_w > 100{,}000$ and 10% OMM2.1.2 (implants type O).

2.4. In vivo drug release

Two types of implants loaded with TA were tested in rabbits ($n = 2$): implant type O, formulated with polymer M_w of 120,000 + 10% oligomer OMM2.1.2 and implant type S, formulated with polymer M_w of 127,000 + 10% Simulsol®. In order to assess the proof of concept, i.e., delivery of TA in the vitreous over months, at least for more than 3 weeks as usually obtained with intravitreal injection of TA (Scholes et al., 1985) (Beer et al., 2003), drug concentrations in ocular structures (mainly vitreous and sclera) have been determined at time points 5 and 8 weeks. A subtenon injection of Kenacort® A40 containing 40 mg TA ($n = 2$) has been used as control. Rabbits were euthanized by an i.v. injection of Phenobarbital 20% (AMINO AG, Neuenhof, Switzerland) 4, 11 and 21 days after implantation. For each type of TA delivery system, both eyes (implanted and contralateral eye) were enucleated. Pieces of cornea, iris and lens were first cut. After ocular ball opening, the whole vitreous was collected. Then retina was peeled off followed by choroid. A piece of sclera around 15 mm^2 was finally cut at the site of implantation/injection. Samples of sclera and vitreous humor were frozen at −80 °C and further processed for HPLC analysis.

2.5. Determination of triamcinolone acetonide concentrations

Biological samples were treated before HPLC analysis. Briefly, tissue samples such as sclera were fragmented using surgical scissors. Extraction of TA was achieved using acetonitrile (2 h vortex) followed by 10 min of centrifugation at 15,000 rpm. The supernatant was then filtered before injection for HPLC run. Treatment of ocular fluids such as vitreous humor was quite similar, diluting 500 μl of biological sample with 500 μl of acetonitrile (5 min vortex followed by centrifugation).

Concentrations of TA have been determined by reversed phase HPLC using LCI Module Plus instrument (Waters, Switzerland). Analysis have been performed with a Nucleosil Column C18 (250 μm × 4.5 μm) at room temperature and using a mixture of water/acetonitrile (60/40) in an isocratic mode as eluant. Flow rate was fixed at 0.8 ml/min and TA was detected by UV detector at 236 nm. Linearity has been demonstrated over the 1–250 μg/ml range. The limits of detection and quantification were, respectively, 0.3 and 0.9 μg/ml.

3. Results and discussion

3.1. Manufacture of scleral implants

Polymer M_w was a parameter of prime importance to manufacture scleral implants having mechanical properties adapted for surgical implantation such as flexibility and resistance. Studying a wide range of M_w (10,000–250,000 Da), it has been observed on one hand that PMM2.1.2 with $M_w < 100{,}000$ lead to the formation of a sticky gel-like formulation. On the other hand, polymer-exhibiting $M_w > 100{,}000$ allowed the fabrication of solid dosage forms such as scleral implants. However, the use of a plasticizer was required to obtain scleral discs with sufficient flexibility, PMM2.1.2 alone leading to the formation of brittling films. Among the several plasticizers tested, best results have been obtained by using either Simulsol® or OMM2.1.2 oligomers. Hence, optimized formulations were made of high M_w PMM2.1.2 (about 120,000) combined with 10% of either Simulsol® or oligomers. Consequently two batches of scleral implants were used for further in vivo TA delivery tests, namely implant type S (Simulsol®) and type O (oligomers). Maximum drug loading was 13.5 ± 0.5% for implants type S and 16.8 ± 0.4% for implant type O, corresponding to 5 and 9 mg of TA, respectively. Scleral implants showed the form of a disc with the following dimensions: 8 mm diameter × 1 mm thickness (Fig. 2). According to the European Pharmacopoeia, THF belongs to the solvents of low toxicity (class 3) with an upper limit at 50 mg/day. Headspace analysis demonstrated that PMM2.1.2. implants were in accordance with these requirements since percentage of THF found in scleral implants was 0.65 ± 0.08% corresponding to 1 mg of THF for the whole implant.

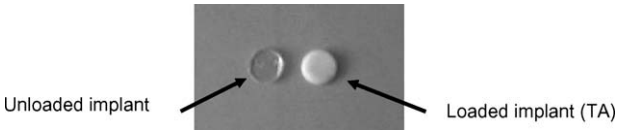

Fig. 2. Loaded and unloaded scleral implant formulated with PMM2.1.2.

It has been observed that the mechanical properties of implants were not affected by gamma irradiations. Furthermore, the sterilization process did not induce qualitative nor quantitative modification of the drug. However, as expected, gamma sterilization induced a reproducible 25% decrease of PMM2.1.2 M_w while the polydispersity remained unchanged. Although the effects of gamma irradiation on properties of biodegradable systems are controversially discussed in literature, it is now most generally accepted that it reduces polymer M_w. This phenomenon is commonly described for numerous polymers such as poly(lactide-co-glycolide) or PLGA (Bittner et al., 1999; Mohr et al., 1999; Hausberger et al., 1995). As example, Bittner et al. (1999) reported a 15% decrease of M_w for PLGA 50/50 having an initial M_w of about 34,000. A similar 15% decrease after gamma sterilization has been recently reported for PMM2.1.2 with M_w of 31,000 (Fournier et al., 2004). The higher degradation observed in our study with PMM2.1.2 can be attributed to the higher initial M_w of the polymer (120,000). Indeed, it has been reported by several authors that high M_w polymers are more sensitive to gamma irradiations than lower ones (Volland et al., 1994; Merkli et al., 1994).

3.2. Biocompatibility

A minimal ocular inflammation could be seen within the first postoperative days resulting from ocular surgery. But inflammation resolved spontaneously in all rabbits. The total clinical score of the implanted eye was ≤1 at day 0, 1, 2 and 3 for each rabbit. In addition, examination of the eye fundus after mydriasis of the pupil did no show any inflammation or retinal detachment at the end of the biocompatibility study.

Anterior chamber puncture performed at the end of the experiment revealed no significant intraocular inflammation, as measured by proteins and inflammatory cells in AH. The total concentration of proteins remained at a low level over a period of 56 days (<1 mg/ml) comparable to the control eye (Fig. 3). Such values are characteristics of an absence of inflammation, since AH of rabbit inflamed eye has been reported to show more than 10 mg/ml of proteins (Rosenbaum et al., 1988; Rubin, 1998), with the lowest reported value at 2.1 mg/ml (Villena et al., 1999).

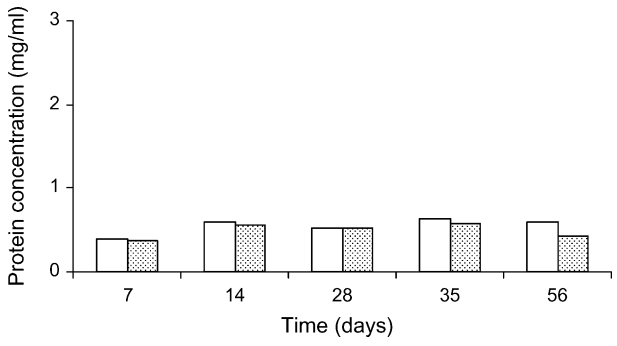

Fig. 3. Concentrations of proteins in rabbit AH at day 7, 14, 28, 35 and 56 after implantation;(□) control eye; (▨) implanted eye (n = 2–3).

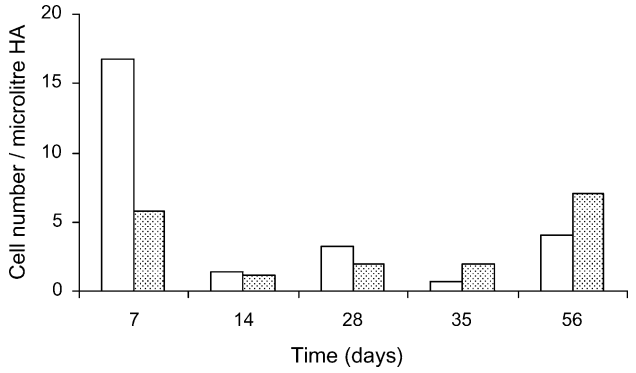

Fig. 4. Number of inflammatory cells in AH of rabbits at day 7, 14, 28, 35 and 56 after implantation;(□) control eye; (▨) implanted eye ($n = 2$–3).

Control non-inflamed rabbit eyes have shown <1 mg/ml proteins in AH (Kadar et al., 2001; McGahan et al., 1996; Villena et al., 1999).

At each time point studied, less than 20 inflammatory cells in AH were counted in implanted and control eyes as well (Fig. 4). This is largely below the levels that are characteristics of inflamed eyes. In fact, in literature control eyes are reported to show between 0 and 276 cells/μl in AH and inflamed eyes 185–3500 cells/μl AH (Rosenbaum et al., 1988; McGahan et al., 1996; Villena et al., 1999).

3.3. In vivo drug release

Both types O and S of scleral implants were easy to handle during surgery. After implantation, scleral implants had adapted to the curvature of ocular ball as shown in Fig. 5.

Concentrations of TA after subtenon injection of control Kenacort® are summarized in Table 2. It demonstrated that repeated injection after 3 weeks is necessary since no more TA can be dosed in the vitreous. TA concentrations gradually increased to reach a maximum concentration at day 11 and totally disappeared at day 21. Moreover, two important observations rose from studying TA release from the control Kenacort®. First, huge amounts of TA are present in the sclera suggesting that this tissue act as a reservoir for lipophilic drugs such as TA. Furthermore, significant concentrations of TA have been measured in the contralateral eye, a phenomenon that has been already described in literature (Bernatchez et al., 1994; Bodker et al., 1993). As an example dexamethasone levels in aqueous and vitreous humors were identical in ipsilateral and contralateral eyes a few hours after subconjunctival or retrobulbal injection (Bodker et al., 1993). On another hand, ophthalmologists observe that, after a subtenon injection of Kenacort®, the contralateral eye shows amelioration. These observations suggest that absorption and delivery of the drug is partially hematogeneous and the drug is redistributed through the systemic blood circulation. This hypothesis is supported by the observation that after subconjunctival injection of prednisolone in rabbit, 2% of the drug was absorbed into ocular tissues and 98% into the systemic circulation (Tsuji et al., 1988).

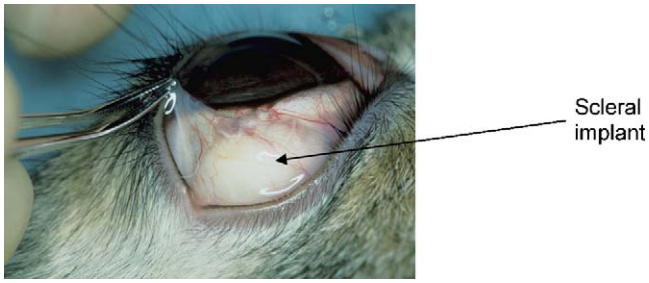

Fig. 5. Scleral implant 5 weeks after implantation.

Table 2
Concentrations of TA in the vitreous humor and the sclera after subtenon injection of Kenacort® (values min–max)

	Injected eye	Contralateral eye
Vitreous (ng/100 μl)		
Day 4	0–22	0
Day 11	37–389	373–413
Day 21	0	0–694
Sclera (ng/mg)		
Day 4	2.6–1710	0–0.58
Day 11	28 800–61 425	0–474
Day 21	0–4220	0

Table 3
TA levels in the rabbit eye 5 weeks after implantation of PMM2.1.2 scleral implants (values min–max)

	Implant type O	Implant type S
Vitreous (ng/100 μl)	0–300	0–935
Sclera (ng/mg)	0–510	39–92

Implant with oligomer OMM2.1.2, type O and implant with Simulsol®, type S.

Determination of TA concentrations released in the vitreous from scleral implants interestingly indicated that it was detectable up to 8 weeks in the vitreous. Therapeutic concentrations were measured up to 5 weeks (Table 3). Hence, this demonstrates that scleral discs based on PMM2.1.2 are able to prolong TA release when compared with injection of Kenacort® suspension.

Furthermore, it has to be noted that scleral implant containing Simulsol® as plasticizer provided higher concentrations of TA in the vitreous (935 ng/100 μl) than those manufactured with oligomers (300 ng/100 μl). This can be explained by the highly hydrophilic nature of Simulsol® when compared with oligomers. Indeed after in vivo insertion of implants, Simulsol® is dissolved by aqueous physiologic media leading to the formation of channels in the PMM2.1.2 matrix, allowing drug release.

For both types of implants, high concentrations of TA were found in the sclera (up to 500 ng/mg of tissue) confirming results obtained with the Kenacort® injection regarding the reservoir effect of the sclera.

4. Conclusions

The compression-molding method was well adapted to obtain scleral implants offering good mechanical properties for in vivo implantation when using PMM2.1.2. of high M_w combined with

suited plasticizer such as oligomers or Simulsol®. The biocompatibility test indicated that PMM2.1.2 based systems do not induce ocular inflammation as demonstrated by the absence of clinical inflammatory signs as well as low levels of proteins and inflammatory cells in AH. Furthermore, encouraging in vivo results have been obtained. Significant concentrations of TA were detected in the vitreous up to 5 weeks after implantation when compared with Kenacort® injections, a result that need to be confirmed by complete pharmacokinetic studies after implantation of PMM2.1.2 scleral implants on a larger population of rabbits.

Finally, this study also demonstrated that the scleral route is promising for the treatment of the posterior segment of the eye, allowing to reaching suited concentrations of drugs within minimizing side effects currently associated with the systemic route.

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