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Research paper

PLGA microspheres for the ocular delivery of a peptide drug, vancomycin using emulsification/spray-drying as the preparation method: in vitro/in vivo studies

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Abstract

The aim of this study was an in vitro/in vivo investigation on poly(lactide-co-glycolide) (PLGA) microspheres as carriers for the topical ocular delivery of a peptide drug vancomycin (VA). The microspheres were prepared by an emulsification/spray-drying technique that can be proposed as an alternative to the double emulsion method for preparation of peptide-loaded microparticles. The drug encapsulation efficiencies were close to the theoretical values (84.2–99.5%); the average particle size, expressed as d_{vs}, was about 11 μm. The microspheres were able to modulate the in vitro drug release of VA with a behavior dependent on their composition: the highest drug content corresponded to the highest release rate. In vivo studies were carried out by assessing the pharmacokinetic profile of VA in the aqueous humor of rabbits after topical administration of aqueous suspensions of microspheres. High and prolonged VA concentrations and increased AUC values (2-fold) with respect to an aqueous solution of the drug were observed. Increasing the viscosity of the microsphere suspension by addition of a suspending-viscosizing agent (hydroxypropylcellulose) did not produce an increase of the ocular bioavailability. PLGA microspheres can be proposed as a system for ocular delivery of peptide drugs.

Keywords: Poly(lactide-co-glycolide); Peptide drug; Vancomycin; Microspheres; Emulsification/spray drying; In vivo studies; Ocular delivery; Aqueous humor

1. Introduction

Endophthalmitis is a severe inflammatory process involving both the anterior and posterior segments of the eye, that can result in permanent eye damage and loss of sight. It can be endogenous, following bacteraemia or fungaemia, or it may occur in post-operative, post-traumatic situations [1]. Endogenous development is usually caused by bacteria such as streptococci. Acute bacterial endophthalmitis represents a true ophthalmological emergency and an effective therapy requires both an immediate and a prolonged treatment with appropriate antibiotics. Intraocular (intravitreal, subconjunctival) injections of vancomycin (VA), a peptide drug, are used [1–3]. Since this drug is poorly absorbed from the gastrointestinal tract, intravenous administration has also been tried. This treatment, however, has been found inadequate to achieve therapeutic levels of VA in the aqueous humor [3]; furthermore, systemic administration of VA can be associated with severe adverse effects [4]. External ocular bacterial infections (conjunctivitis, blepharitis, etc.) are also conditions constituting a serious risk. The infecting organism is most often *Staphylococcus aureus*, but other bacteria (e.g. *Pseudomonas aeruginosa*) can be responsible. In the case of these external infections, VA, although showing a high antibacterial activity to *S. aureus* and other staphylococcal species [5], is not currently used in topical ocular therapy, since its high molecular weight and elevated

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hydrophilicity preclude a prolonged retention in the external eye structures and a satisfactory corneal penetration.

Alternative formulations to traditional aqueous eyedrops might improve the ocular performance of this antibiotic. Microparticulate polymeric delivery systems (microspheres, microcapsules, etc.) have been investigated as a possible approach to increase the ocular drug availability [6–8].

In the present investigation, VA was incorporated in poly(lactide-co-glycolide) (PLGA) microspheres, with the aim of improving both its preocular permanence and its corneal penetration, hence its efficacy against external/internal ocular bacterial infections. Polylactide (PLA) and PLGA are polymers widely used in the preparation of microparticles, since they are non-toxic, well tolerated by the human body, biodegradable and biocompatible [9–11]. However loading of peptides as VA into PLA and PLGA microparticles can be problematic owing to their high hydrophilicity. The most used technique to encapsulate proteins is the double (w/o/w) emulsification method, followed by solvent evaporation; this method is reported in the literature for preparation of VA-loaded microparticles for possible intratecal use [12].

We prepared VA-loaded PLGA microspheres using an emulsification/spray-drying method, which is proposed as an alternative to double emulsification. A single w/o emulsion, containing VA dissolved in the aqueous dispersed phase and PLGA dissolved in the organic continuous phase, was spray-dried.

The microparticles obtained were characterized in terms of shape (SEM microscopy), size (laser diffraction method) and drug loading. Their in vitro release behaviour was investigated in phosphate buffer (pH 7.0). As preliminary biological study, aqueous suspensions of VA-loaded microspheres, with or without a suspending-viscosizing agent (hydroxypropylcellulose, HCP) were evaluated in vivo in rabbits determining the drug level in the aqueous humor, using as references two VA aqueous solutions (simple and viscosized).

2. Materials and methods

2.1. Materials

Vancomycin hydrochloride (VA) was kindly given by Eli Lilly Italia S.p.A. (Sesto Fiorentino, Firenze, Italy); poly(D,L-lactide-co-glycolide) (PGLA, Resomer RG502H, inherent viscosity 0.16 dl/g) was purchased from Boehringer Ingelheim KG (Ingelheim/Rhein, Germany); hydroxypropylcellulose (HPC), type HF pharm was purchased from Hercules Italia S.p.A., Aqualon Division (Bologna, Italy); potassium dihydrogen phosphate RPH and acetic acid RPE were obtained from Carlo Erba Srl, (Milano, Italy); sodium acetate ACS reagent and acetonitrile (CHROMASOLV® for HPLC, gradient grade) were purchased from Rielden-de Haën AG, (Seelze, Germany); phosphoric acid ACS reagent and Tween® 80 were supplied by Sigma-Aldrich Chemie GmbH, (Steinheim, Germany); PTFE syringe filters 13 mm, 0.45 μm porosity were provided by Alltech Italia Srl, (Sedriano, Milano, Italy).

All other solvents and chemicals were of analytical grade.

2.2. Preparation of the microspheres (emulsification/spray-drying technique)

VA-loaded microspheres with three different drug/polymer ratios (VA1–VA3) were prepared. Their compositions are reported in Table 1. The first step was the preparation of a w/o emulsion. VA was dissolved in 10 ml of distilled water and PLGA in 290 ml of dichloromethane. The amounts of drug and polymer were different depending on the composition of the microspheres to be prepared. The total amount of solid (drug and polymer) was always 3 g. The amount of polymer for each formulation was 2.4, 2.25 and 2.0 g and the amount of drug was 0.6, 0.75 and 1.0 g for (VA1), (VA2) and (VA3), respectively. The aqueous drug solution was added dropwise to the polymer solution within 5 min, under stirring at 10,000 rev./min with a homogenizer (Ultra-Turrax® T25 basic, IKA, Germany). The temperature was maintained at about 5 °C throughout the process. The second step consisted of spraying the w/o emulsion through the nozzle (0.7 mm) of a spray-dryer, co-current flow type (model Mini Spray HO Pabisch, W.Pabisch S.p.A., Milano, Italy). The process conditions were: inlet-air temperature 80–85 °C; outlet-air temperature 68–70 °C; spray-pressure about 203 kPa, spray rate of feed about 10 ml/min. The emulsion was kept at a temperature of about 5 °C, under magnetic stirring, during the spraying process. The total volume of the emulsion used for the preparation of each batch was 300 ml. No phase separation in the emulsion during the spray-drying process was observed, thus rendering unnecessary the use of surfactants. The solid microspheres were collected and kept under vacuum for 48 h at room temperature. Drug-free microspheres (VA0) were prepared by spraying a w/o emulsion of PLGA (1% w/v) by the same method used for drug-loaded microspheres.

Table 1
Theoretical composition, drug content, encapsulation efficiency and particle size of spray-dried microspheres ($n = 3$)

Microspheres	Theoretical composition (%)		Actual drug content		Encapsulation efficiency (%)	Particle size	
	PLGA	VA	%	SD		d_{vs}, μm	SD
VA1	80.0	20.0	19.9	1.77	99.5	11.75	1.31
VA2	75.0	25.0	21.5	0.37	86.0	10.96	0.08
VA3	66.7	33.3	27.4	0.93	84.2	11.15	0.69
VA0	100.0	0	–	–	–	7.72	1.15

The conditions were as follows: inlet-air temperature about 75 °C; outlet-air temperature 50–52 °C; spray-pressure about 203 kPa and spray rate of feed about 15 ml/min. To verify the reproducibility of the methods, each preparation was carried out in triplicate, using the same experimental conditions (SD within about 3%).

2.3. Scanning electron microscopy

The morphology of the microspheres was examined using a scanning electron microscope (SEM, Zeiss DSM 962, Zeiss, Germany); images of VA1 microspheres were also taken after the in vitro drug release tests. Samples of microspheres were placed on double-sided tapes which had previously been secured on aluminum stubs and then analyzed after gold sputtering at 20 kV acceleration voltage, under an argon atmosphere.

2.4. Particle size analysis

Particles were sized by the light diffraction method using a Coulter apparatus, model LS 100Q (Beckman Coulter Particle Characterization, Miami, FL). Particle size analyses were carried out on blank and drug-loaded spray-dried microspheres that were suspended in water (0.1% w/v Tween® 80) and sonicated for 10 s. Three analyses were performed for each microsphere sample. The average particle size of each sample was expressed as the volume-surface diameter (d_{vs}, μm) [13].

2.5. Determination of vancomycin content

Drug loaded microspheres (20 mg) were dissolved in dichloromethane (1.0 ml), and VA was extracted with diluted phosphoric acid (pH 4.9). After sonication (Sonorex RK 52H, Bandelin Electronic, Berlin, Germany) for 5 min at 25 °C, the organic solvent was evaporated under vacuum. The aqueous phase was transferred into a volumetric flask and acid solution was added up to 50 ml. One millilitre of the dispersion was centrifuged for 5 min at 13,000 rev./min (Microfuge® Lite, Beckman, Germany) and the supernatant was analyzed by HPLC after filtration (0.45 μm filters). Each determination was carried out in triplicate.

2.6. In vitro vancomycin dissolution and release studies

In vitro dissolution and release tests on VA-loaded microspheres and on VA powder (pure drug) were carried out using a USP dissolution apparatus n.1 (Erweka DT 70, Erweka GmbH, Heusenstamm, Germany) on about 10 mg VA or on samples of microspheres corresponding to about 10 mg of VA. The tests were performed using USP phosphate buffer as medium (pH 7.0, 500 ml, 37 °C, 50 rev./min). Aliquots (1.0 ml) of the medium were withdrawn at preset times by an automatic sampling system (Erweka DT 70, Erweka GmbH, Heusenstamm, Germany), filtered (0.45 μm) and analyzed by HPLC using the apparatus and the conditions described below. An equal volume of fresh medium was added after each sampling. Each experiment was performed in triplicate.

2.7. HPLC analysis

The equipment consisted of an HPLC system including an autosampler (Hewlett-Packard 1050 Series), quaternary pump thermostat, variable-wavelength UV–Vis spectrophotometer detector (all from Hewlett-Packard, Waldbronn, Germany). The peak areas determined with a 3390 integrator (Hewlett-Packard, Avondale, PA, USA) were used for quantitation. Reverse-phase HPLC was performed at room temperature. The analytical column (250 × 4.6 mm I.D.) was packed with Adsorbosil ODS C18 (5 μm) preceded by a Nucleosil RP-18 (5 μm) (Alltech Italia Srl, (Sedriano, Milano, Italy). Twenty microlitres of samples or calibration standards were injected directly into the column and were eluted with a gradient consisting of phosphate buffer (pH 2.8) (A) and acetonitrile (B). Zero-time conditions were A:B (90:10). After 5 min, the gradient was adjusted to A:B (60:40), until termination of the run at 10 min, the flow rate was 1 ml/min. The system was equilibrated for 10 min under the starting conditions before injecting the next sample. Detection was carried out by monitoring the absorbance at 280 nm (0.1 AUFS). The mobile phases were filtered with a 0.45 μm pore size cellulose membrane filter, and degassed before use. The pH of the buffer was adjusted to 2.8 with phosphoric acid. Each determination was carried out in triplicate (SD within 0.08).

2.8. In vivo studies

2.8.1. Animals

Male New Zeland albino rabbits, free of ocular defects, weighing approximately 2.8–3.5 kg (Pampaloni rabbitry, Fauglia, Italy) were used and treated as prescribed in the publication *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 92–93, revised 1985). The in vivo experimental protocol was approved by the Ethical–Scientific Committee of the University of Pisa and all experiments were carried out under veterinary supervision.

The animals were housed singly in standard cages, in a light-controlled room (10:14 h dark/light cycle) at 19 ± 1 °C and 50 ± 5% RH, and had no restriction of food and water. During the in vivo experiments the rabbits were placed in restraining boxes: they were allowed to move the head freely, and their eye movements were not restricted.

2.8.2. Vancomycin concentration profiles in the aqueous humor

The following preparations, all containing 10 mg/ml VA were tested (VA_{micr}): 5.03% w/w suspension of VA1

microspheres in phosphate buffer (pH 7.4); (VA_{micr}–HPC): 5.03% w/w aqueous suspension of VA1 microspheres in 0.5% w/w HPC (Newtonian viscosity, 26.0 MPa s); VA_{sol}: VA solution in phosphate buffer (pH 7.4); VA_{sol}–HPC: VA solution in phosphate buffer (pH 7.4), viscosized with 0.5% w/w HPC. The osmolality of all preparations was adjusted to physiological values by adding appropriate amounts of sodium chloride.

The in vivo tests were carried out by instilling 100 μl (2×50 μl drops at 90 s intervals) of each suspension or solution immediately after their preparation into the lower conjunctival sac of one eye of each rabbit; at least six rabbits were used for each time point.

At appropriate times after administration, the rabbits were anaesthetized by i.m. administration of 30 mg/kg ketamine (Inoketam 1000 solution, Virbac Srl, France) and 5 mg/kg xylazine (Rompum 2% solution; Bayer AG, Leverkusen, Germany). After a few minutes, 50–80 μl of aqueous humor were aspirated from the anterior chamber using a 1.0-ml insulin syringe fitted with a 29-gauge needle (B-D, Micro-Fine U-40 insulin; Beckton Dickinson, Dublin, Ireland). The aqueous humor samples were immediately frozen and stored at −18 °C. For analysis the samples were mixed with an equal volume of methanol containing 6% v/v perchloric acid; after centrifugation (3 min at 12,000 rev./min; Microfuge 11, Beckman Instruments, Palo Alto, CA, USA), 20 μl of the supernatant were submitted to HPLC analysis.

2.8.3. Pharmacokinetics analysis

The AUC values (areas under the concentration vs. time curves) for VA in aqueous humor were calculated from the beginning (t_0) to the end of the observation time (t_{last}), from appropriate graphs using the linear trapezoidal rule (Kaleidagraph, Synergy Software).

2.8.4. Statistical data analysis

The statistical significance of the differences between means (for pharmacokinetic data) was first tested by one-way analysis of variance (ANOVA test, Statview Software, Abacus Concepts, Inc., Berkeley, CA). When differences were detected, multiple comparisons were performed among the data corresponding to different dosing times using the Fisher protected least significant difference (PLSD) test.

3. Results and discussion

The emulsification/spray-drying technique described here appears to be a suitable method for the preparation of PLGA microspheres loaded with a water soluble peptide drug (VA). The technique is rapid and involves preparation of a simple (w/o) emulsion in which the drug is dissolved in the aqueous phase, while the polymer is dissolved in the continuous organic phase. The emulsion (kept at about 5 °C) maintains its stability until the end of the spray-drying process, thus avoiding the use of surfactants. Emulsification/spray-drying can be proposed as an alternative to the double (w/o/w) emulsification method, a technique commonly used to entrap water-soluble drugs in hydrophobic polymers [14]. Nevertheless, many factors such as the solubility of the polymer and the type and concentration of the emulsifier can affect the final properties of the resulting microparticles.

The compositions and characteristics of the spray-dried microspheres are reported in Table 1.

The reproducibility of the spray-drying method is good, as indicated by drug content and encapsulation efficiency determinations carried out on each group of three batches prepared under identical conditions (SD within 1.77). Drug contents are always close to the theoretical values and good encapsulation efficiencies are obtained. Mean encapsulation efficiencies, always high, decrease with increasing drug-to-polymer weight ratios. In fact, an almost total encapsulation is obtained in microspheres with VA-to-PLGA weight ratio 1:4, while 86% and about 84% encapsulation efficiency values are observed for VA-to-PLGA weight ratios 1:3 and 1:2, respectively. Independently of the drug-to-polymer weight ratio, the production yields (expressed as % w/w of the initial amounts of drug and polymer) are relatively low (no values above 50–55%). As pointed out previously for other spray-dried preparations [6], this result can be attributed mainly to the loss of the smallest and lightest microparticles, exhausted by the aspirator of the spray-dryer apparatus during the process and also to the small amount of materials processed in each batch (3 g). Similar low production yields are also observed for drug-free microparticles.

SEM photomicrograph of PLGA drug-loaded microspheres (Fig. 1, VA1 batch, chosen as an example) shows almost spherical structures with smooth surfaces. The formation of some agglomerates is observed when the drug-to-polymer weight ratio is increased. No free drug crystals mixed with microspheres are present, indicating

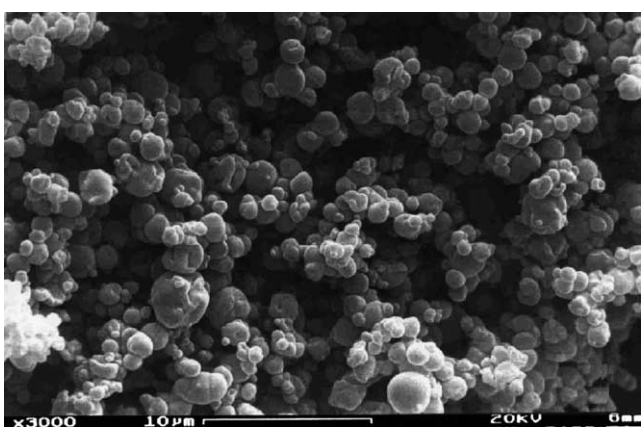

Fig. 1. Morphological appearance of PLGA loaded microspheres (VA1: drug-to-polymer ratio 1:4, chosen as an example).

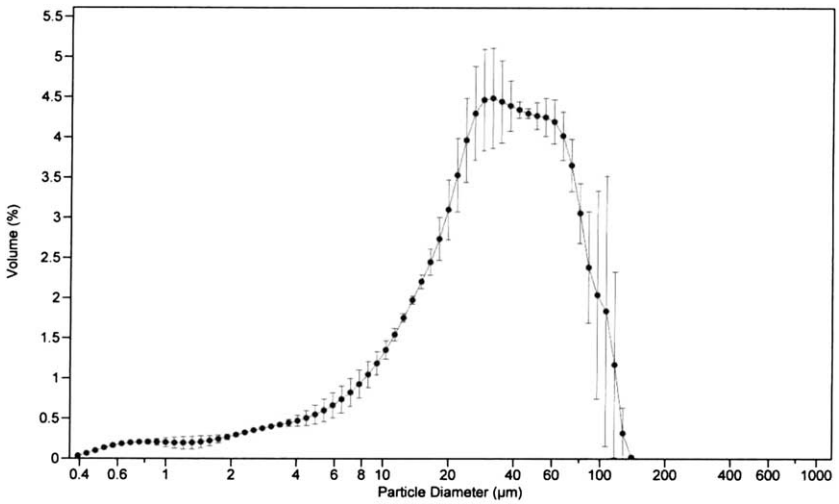

Fig. 2. Differential particle size distribution by volume of VA-loaded microparticles, VA1 (each curve results from the mean of three measurements).

complete loading of VA in the PLGA micromatrix. Empty microparticles (VA0), prepared as a comparison, show similar morphological characteristics.

Microparticulate systems for ophthalmic use must be extremely small-sized to avoid ocular damage due to abrasion and irritation. As shown in Table 1, laser diffraction measurements indicate for drug-loaded microparticles an average particle size, expressed as d_{vs} (diameter volume-surface), of about 11 μm. This indicates that the different drug-to-polymer weight ratios used in these preparations did not substantially influence the size of spray-dried particles. The particle size distribution curve by volume of VA1, chosen as an example, is shown in Fig. 2.

Drug-free microparticles (VA0) are characterized by the lowest size (d_{vs} about 7.72 μm).

The in vitro VA release profiles from drug-loaded microspheres are presented in Fig. 3, in comparison with the dissolution profile of VA powder (pure drug). The rate of dissolution of VA powder is quite fast: more than 80% drug is dissolved in about 5 min. VA loading of VA into the microspheres leads to a modulation of in vitro drug release, depending on their composition. The highest drug contents (VA3, 27.4% and VA2, 21.5%) correspond to the highest release rates, the lowest release rate corresponds to the lowest drug content (VA1, 19.9%).

This result might also be explained assuming that the release of the high hydrophilic VA (hydrochloride) from the PLGA matrix of the microparticles occurs by diffusion, a process strictly dependent on drug concentration. This is confirmed by SEM images of microspheres taken after the drug release tests, which show almost intact microparticles (pictures not reported).

The results obtained from the in vivo tests are shown in Fig. 4, illustrating VA levels in the aqueous humor of rabbits after administration of the VA_{micr} and VA_{micr}–HPC microsphere suspensions and of the two reference solutions, VA_{sol} and VA_{sol}–HPC. The relevant pharmacokinetic parameters, VA concentration peak (C_{max}, μg/ml), T_{max} (min) and area under the concentration vs. time curve (AUC, min μg/ml), are reported in Table 2.

The preparation VA_{micr} (prepared with microspheres characterized by a VA-to-PLGA weight ratio 1:4) increases the drug concentration in the aqueous humor when compared to the two reference solutions, VA_{sol} and VA_{sol}–HPC. The AUC value of VA_{micr} is more than two-fold higher with respect to those of VA_{sol} and VA_{sol}–HPC (Table 2). As shown in Fig. 4, 15 min after administration of the preparation VA_{micr} a significantly ($P < 0.05$) higher VA concentration in the aqueous is reached with respect to both reference solutions ($C_{15\ min}$ 2.47, 0.80 and 1.26 μg/ml for VA_{micr}, VA_{sol} and VA_{sol}–HPC, respectively). The VA concentration remains higher with respect to reference solutions for a prolonged time, with statistically significant differences ($P < 0.05$) 120 and 150 min after administration.

The preparation VA_{micr}–HPC, viscosized with HPC, shows a lower C_{max} value with respect to VA_{micr}

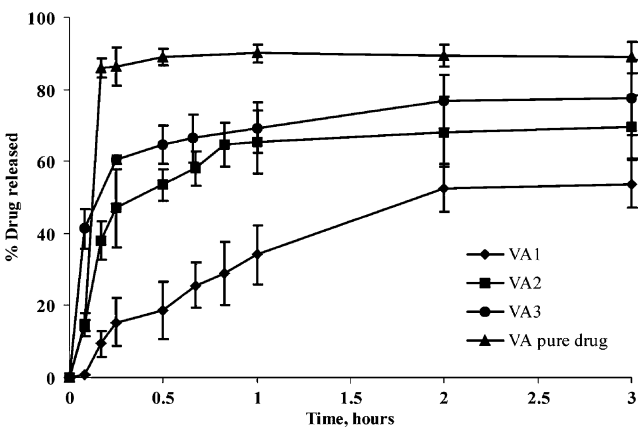

Fig. 3. In vitro release profiles of drug-loaded microspheres compared to the dissolution profile of the pure drug.

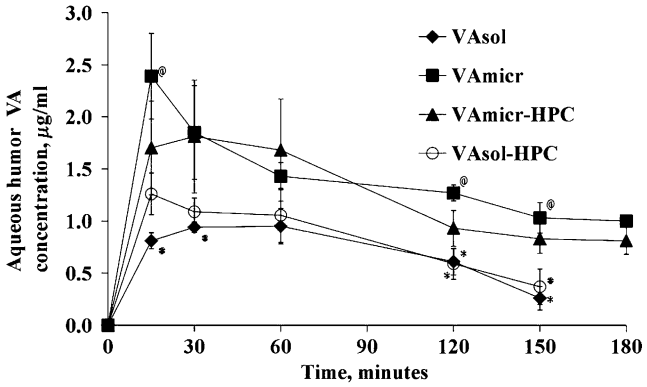

Fig. 4. VA concentration profiles in the aqueous humor of rabbits after administration of VA_{micr} and VA_{micr}–HPC microsphere suspensions and of the reference solutions (VA_{sol} and VA_{sol}–HPC) (Mean ± SE, $n = 6$; *significantly different from the VA_{micr} and the VA_{micr}–HPC formulations, $P < 0.05$, #significantly different from the VA_{micr}–HPC formulation, $P < 0.05$).

(1.80 and 2.47 μg/ml, respectively for VA_{micr}–HPC and VA_{micr}), and lower VA concentration in the aqueous humor 120 min after administration. However, as show in Table 2, VA_{micr} and VA_{micr}–HPC suspensions show very similar bioavailabilities. The two reference solutions VA_{sol} and VA_{sol}–HPC (without and with HPC as viscosizing agent, respectively) are also characterized by similar AUC values, about 107 and 123 min μg/ml, respectively (Table 2), thus indicating that the presence of HPC does not lead to any bioavailability improvement. This result is not foreseen, in view of the known effect of vehicle viscosity on ocular bioavailability [15], and it might be explained on the basis of the rapid ocular clearance of the drug, due to its high hydrophilicity, and of the low sensitivity of rabbits to vehicle viscosity effects [16].

In conclusion, of the four formulations tested , only VA_{micr} and VA_{micr}–HPC are capable of maintaining appreciable VA concentrations in the aqueous humor 180 min after administration (0.85 and 0.81 μg/ml, respectively). All preparations are well tolerated by the rabbits: in no case were signs of irritation or ocular discomfort observed.

On the basis of these preliminary results, spray-dried PLGA microspheres can be proposed as a topical ocular delivery system for peptide drugs such as VA. The microparticles can be formulated as suspensions: addition of HPC as a viscosity-increasing agent and suspension stabilizer, while improving the physical stability of the suspension, does not appear to influence the in vivo behavior of the preparations, at least on the rabbit model tested in this investigation. As shown by the comparison with the reference VA solutions the microparticles improve the aqueous humor bioavailability of the hydrophilic peptide drug: a possible explanation for this effect might reside in a prolonged retention in the cul-de-sac and precorneal area, due to the small size, and in a gradual release of the entrapped peptide to the absorbing corneal surface.

Table 2
Pharmacokinetic parameters in aqueous humor after in vivo administration in rabbits of the preparations under study

Preparation	C_{max} (μg/ml ± SE)	T_{max} (min)	AUC (min μg/ml ± SE)	AUC relative
VA_{micr}	2.47 ± 0.49	15	248.2 ± 35.2	2.31
VA_{micr}–HPC	1.80 ± 0.54	30	206.6 ± 52.8	1.92
VA_{sol}	0.94 ± 0.05	30	107.4 ± 17.3	1.00
VA_{sol}–HPC	1.26 ± 0.21	15	122.9 ± 26.7	1.14

References

[1] D. Brod, H.W. Flynn, Infectious endophthalmitis, Curr. Opin. Infect. Dis. 10 (1997) 153–162.
[2] S.C. Ptlughelder, H.W. Flynn, Infectious endophthalmitis, Infect. Dis. Clin. North Am. 6 (1992) 859–873.
[3] M. Souli, G. Kopsinis, E. Kavouklis, L. Gabriel, H. Giamarellou, Vancomycin levels in human aqueous humour after intravenous and subconjunctival administration, Int. J. Antimicrob. Agents 18 (2001) 239–243.
[4] J.E.F. Reynolds, Martindale The Extra Pharmacopoeia, 31st ed., Pharmaceutical Press, London, UK, 1996, pp. 295–297.
[5] R.P.F. Cheung, J.T. Dipiro, Vancomycin; an update, Pharmacotherapy 6 (1986) 153–169.
[6] P. Giunchedi, U. Conte, P. Chetoni, M.F. Saettone, Pectin microspheres as ophthalmic carriers for Piroxicam: evaluation in vitro and in vivo in albino rabbits, Eur. J. Pharm. Sci. 9 (1999) 1–7.
[7] P. Giunchedi, P. Chetoni, U. Conte, M.F. Saettone, Albumin microspheres for ocular delivery of Piroxicam, Pharm. Pharmacol. Commun. 6 (2000) 149–153.
[8] M.K. Yeh, S.M. Tung, D.W. Lu, J.L. Chen, C.H. Chiang, Formulation factors for preparing ocular biodegradable delivery system of 5-fluorouracil microparticles, J. Microencapsul. 18 (2001) 507–519.
[9] A.A. Ignatius, L.E. Claes, In vitro biocompatibility of bioerosorbable polymers: poly(D,L-lactide) (PLA) and poly(D,L-lactide-co-glicolide) (PLGA), Biomaterials 17 (1996) 831–839.
[10] J. Mordenti, K. Thomsen, V. Licko, L. Berleau, J.W. Kahn, R.A. Cuthbertson, E.T. Duenas, A.M. Ryan, C. Schofield, T.W. Berger, Y.G. Meng, J. Cleland, Intraocular pharmacokinetics and safety of a humanized monoclonal antibody in rabbits after intravitreal administration of a solution or a PLGA microsphere formulation, Toxicol. Sci. 52 (1999) 101–106.
[11] H. Pinstner, R. Gutwald, R. Ordung, J. Reuther, J. Muhling, Poly(L-lactide): a long term degradation study in vivo, Biomaterials 14 (1993) 671–677.
[12] Y. Özalp, N. Özdemir, S. Kocagöz, V. Hasirci, Controlled release of Vancomycin from biodegradable microcapsules, J. Microencapsul. 18 (2001) 89–110.
[13] C. Edmunson, Particle-size analysis, in: H.S. Bean, J.E. Carless, A.H. Beckett (Eds.), Advances in Pharmaceutical Sciences, Vol. 2, Academic Press, New York, 1967, pp. 95–179.
[14] P.K. Gupta, H. Johnson, C. Allexon, In vitro and in vivo evaluation of clarithromycin/poly(lactic acid) microspheres for intramuscular drug delivery, J. Controlled Release 26 (1993) 229–234.
[15] O. Olejnik, Conventional systems in ophthalmic drug delivery, in: A.K. Mitra (Ed.), Ophthalmic Drug Delivery Systems, Marcel Dekker, New York, 1993, pp. 177–198.
[16] M.F. Saettone, B. Giannaccini, F. Barattini, N. Tellini, The validity of rabbits for investigations on ophthalmic vehicles: a comparison of four different vehicles containing tropicamide in humans and rabbits, Pharm. Acta Helv. 57 (1982) 47–55.