ELSEVIER

International Journal of Pharmaceutics 238 (2002) 241–245

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin

Roberta Cavalli [a,*], M. Rosa Gasco [a], Patrizia Chetoni [b], Susi Burgalassi [b], M. Fabrizio Saettone [b]

[a] *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, I-10125, Torino, Italy*
[b] *Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, I-56126, Pisa, Italy*

Received 1 August 2001; received in revised form 7 January 2002; accepted 27 February 2002

Abstract

Aim of this study was to evaluate solid lipid nanoparticles (SLN) as carriers for topical ocular delivery of tobramycin (TOB). The SLN were in the colloidal size range (average diameter below 100 nm; polydispersity index below 0.2) and contained 2.5% TOB as ion-pair complex with hexadecyl phosphate. The preocular retention of SLN in rabbit eyes was tested using drug-free, fluorescent SLN (F-SLN): these were retained for longer times on the corneal surface and in the conjunctival sac when compared with an aqueous fluorescent solution. A suspension of TOB-loaded SLN (TOB-SLN) containing 0.3% w/v TOB was administered topically to rabbits, and the aqueous humour concentration of TOB was determined up to six hours. When compared with an equal dose of TOB administered by standard commercial eyedrops, TOB-SLN produced a significantly higher TOB bioavailability in the aqueous humour.

Keywords: Solid lipid nanoparticles; SLN; Tobramycin; Rabbits; Preocular retention; Ocular delivery; Aqueous humour bioavailability

Solid lipid nanoparticles (SLN) represent an interesting alternative to traditional colloidal carriers, such as emulsions, liposomes and polymeric nanoparticles. In recent years, many hydrophobic and hydrophilic drugs such as e.g. nifedipine, diazepam, desoxycorticosterone, cyclodextrin complexes with hydrocortisone and progesterone, doxorubicin, paclitaxel, tobramycin, timolol, pilocarpine, etc. have been incorporated into SLN (Gasco, 1997, 2001), and administration of SLN by different routes (parenteral, oral, ocular, etc.) has been investigated (Siekman and Westesen, 1992; Domb, 1995; Yang et al., 1999; Müller et al., 2000).

We reported previously on the improved ocular bioavailability in rabbits of pilocarpine incorporated as ion-pair into SLN (Cavalli et al., 1995). Further studies on SLN have evidenced the capacity of these vectors to facilitate gastrointestinal absorption of tobramycin (TOB), while TOB and

* Corresponding author. Tel.: +39-011-6707668; fax: +39-011-6707687.
E-mail address: roberta.cavalli@unito.it (R. Cavalli).

0378-5173/02/$ - see front matter
PII: S0378-5173(02)00080-7

other aminoglycosidic drugs in conventional formulations are generally not absorbed by this route (Cavalli et al., 2000; Bargoni et al., 2001). TOB-loaded liposomes have been reported to be more effective than the free drug in subconjunctival (Assil et al., 1991) and topical (Frucht-Perry et al., 1992) treatment of experimental bacterial keratitis in rabbits. Thus, the aim of this study was to verify if topical instillation of TOB-loaded SLN would improve the ocular bioavailability of the drug with respect to standard eyedrops. The residence time of SLN in rabbits eyes was determined using SLN marked with a fluorescent probe, and the TOB concentration profile in the aqueous humour was determined after topical administration to rabbits of SLN containing TOB as ion complex with hexadecyl phosphate.

Stearic acid was from Fluka (Buchs, CH); Epikuron 200 (soya phosphatidylcholine 95%) was a kind gift from Lucas Meyer (Hamburg, D); tobramycin base (TOB) and fluorescamine were from Sigma (St. Louis, MI, USA); sodium taurocholate was a kind gift from PCA (Basaluzzo, I); coumarin 6, laser grade, [3-(2′-benzotiazolyl)-7-diethylaminocoumarin] was from Carlo Erba (Milan, I). Cremophor® EL (polyethoxylated castor oil, average MW 2513) was from BASF (Ludwigshafen, D). Sodium hexadecyl phosphate was prepared as indicated by Brown (10). The other chemicals and solvents were of analytical grade. Tobral® eyedrops (tobramycin 0.3%, Alcon Laboratories Inc. Forth Worth, TX, USA) were used as reference.

SLN containing the ion-pair complex of TOB with hexadecyl phosphate (TOB-SLN; drug:hexadecyl phosphate 1:2 molar ratio) were prepared by the the warm o/w microemulsion method described elsewhere (Cavalli et al., 1999). Briefly, the microemulsion consisted of stearic acid (internal phase, 0.7 mmol), Epikuron 200 (surfactant, 0.14 mmol), sodium taurocholate (co-surfactant, 0.69 mmol) water (111.1 mmol) and TOB (0.026 mmol) as ion-pair complex. SLN were obtained by dispersing the warm microemulsion in filtered cold water (2–3 °C) at a 1:5 microemulsion:water (v/v) ratio under mechanical stirring.

The SLN aqueous dispersion was washed three times by diaultrafiltration (Diaflo YM 100 membrane, cut off MW 100 000 Da); then was freeze-dried for quantitative determination of the incorporated drug. The taurocholate found in the three washing waters (HPLC analysis) was over 70% of the initial amount. For the 'in vivo' experiments, freeze-dried TOB-SLN were dispersed in pH 7.4 isotonic phosphate buffer to a final 0.3% w/v TOB concentration; the dispersion was then sterilised with saturated steam for 15 min at 121 °C.

Fluorescent SLN (denominated F-SLN) were prepared by adding 3.4×10^3 mmol of coumarin 6 to the melted internal phase of the drug-free microemulsion. The F-SLN were then processed and freeze-dried as indicated above. For the preocular retention study, a 3.0% w/w dispersion of F-SLN in pH 7.4 isotonic phosphate buffer was used.

The quantitative determination of TOB was performed on the freeze-dried TOB-SLN by reversed phase HPLC, after dissolution in methanol and derivatization with fluorescamine (Walker and Coates, 1981), using a fluorescence detector ($\lambda_{ex} = 424$ nm and $\lambda_{em} = 476$ nm). The percent TOB incorporated into TOB-SLN was 2.50% w/w. The average diameter and polydispersity index of SLN were determined at 25 °C by dynamic light scattering (90 Plus particle sizer, Brookhaven Instrument Corp., NY, USA) at a fixed angle of 90°. Each measurement was repeated at least ten times.

All biological tests were carried out on male, New Zealand albino rabbits, weighing 2.8–3.5 kg (Pampaloni rabbitry, Fauglia, Italy). The experiments were carried out under veterinary supervision, and the protocols were approved by the ethical-scientific committee of the University of Pisa. The animals were housed singly in standard cages, in a light-controlled room (10 h dark/14 h light cycle) at 19 ± 1 °C and 50 ± 5% R.H., with no restriction of food or water. During the experiments the rabbits were placed in restraining boxes and their eye movements were not restricted.

The preocular retention of SLN was investigated as described in previous papers (Saettone et

al., 1991; Giunchedi et al., 1999) by instilling 50 μl (2 × 25 μl, at 90 s intervals) of the F-SLN dispersion into the lower conjunctival sac of rabbit eyes. The corneal area was then examined at intervals with a slit-lamp fitted with a blue filter. As reference was used an aqueous dispersion of coumarin 6 solubilized with 5% w/v Cremophor EL (denominated F-SOL), whose coumarin molar concentration was equivalent to that in the F-SLN dispersion. Each preparation was tested in one eye of at least six different rabbits, and the time course of fluorescence over the cornea and in the conjunctival sac was noted always by the same operator.

The ocular bioavailability study was carried out by administering in the lower conjunctival sac of one eye of each rabbit 100 μl (2 × 50 μl, at 90 s intervals) of the TOB-SLN formulation or of the reference eyedrops. The same amount of TOB (0.3 mg) was administered in both cases. At least six rabbits (six eyes) were used for each time point and for each formulation. At appropriate time intervals, the rabbits were euthanized and 75–100 μl samples of aqueous humour were aspirated from the anterior chamber. Fifty microliters of each sample were treated with 200 μl of acetonitrile to precipitate proteins and then centrifuged at 6000 *g* for 5 min. One hundred and fifty microliters of a fluorescamine solution (1.2 mg/ml) in acetonitrile and 700 μl of pH 8.0 phosphate buffer were added to 150 μl of surnatant. After 1 h the samples were analysed by HPLC

The ocular tolerance and potential irritation of the SLN dispersions were separately tested on at least three rabbits, by instilling 50 μl in one eye, while the other eye (reference) was treated with 50 μl of physiological saline. All eyes were examined by slit-lamp 0.5, 1.0, 3.0 and 12 h after treatment, and the irritation was evaluated according to a scoring scale described in a previous paper (Bottari et al., 1978).

Some essential physico-chemical characteristics of the TOB-SLN and of the F-SLN are reported in Table 1.

The results of the pre-ocular retention study (fluorescence in conjunctival sac and on corneal surface) of F-SLN and of the reference F-SOL solution are reported in Table 2. The F-SLN dispersion formed a stabler precorneal film and was retained longer in the eye, while the fluorescent solution formed a short-lived, weakly fluorescent film on the corneal surface and disappeared rapidly from the eye surface.

Table 1
Essential physico-chemical characteristics of the SLN used in the study

	Average diameter (nm)	Polydispersity index	Coumarin 6 incorporated (%w/w)	TOB incorporated (%w/w)
TOB-SLN	80	0.12	–	2.5
F- SLN	70	0.15	0.3	–

Table 2
Results of the preocular retention study of F-SLN

	Time (min)	fluorescence in conjunctival sac	fluorescence on cornea
F-SLN	15	Present	Uniform film
	30	Present	Thin film after blinking
	60	Present	Absent
	90	Low	–
	120	Absent	–
F-SOL	15	Present	Thin film after blinking
	30	Low	Absent
	60	Absent	–

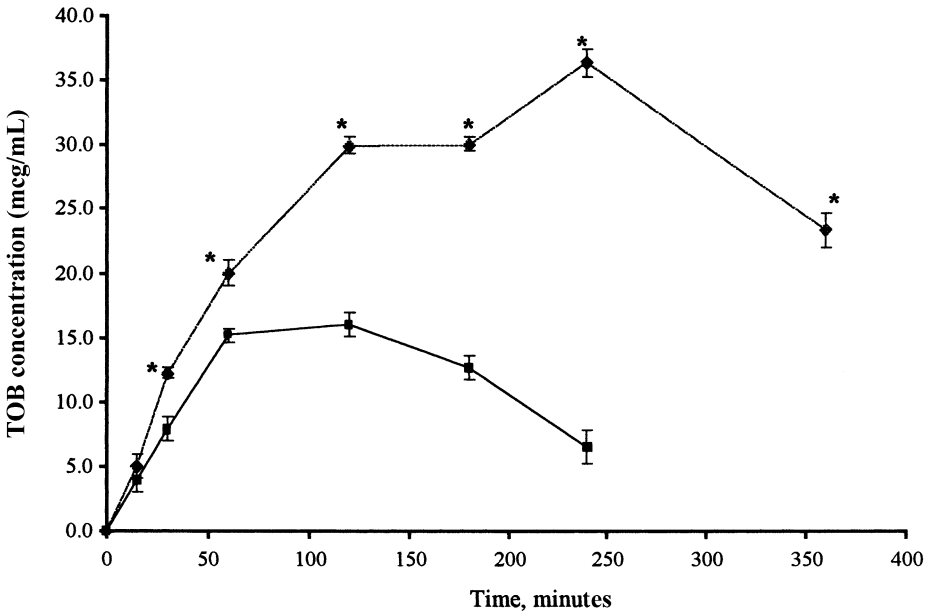

Fig. 1. TOB concentration profiles in aqueous humour after administration of the reference solution (■) and of TOB-SLN (◆). Means ± S.E., $n = 6$.

Table 3
Aqueous humour pharmacokinetic parameters of the formulations under study

Formulation	C_{max} (μg· ml^{-1} (± S.E.))	T_{max} (h)	AUC[a] (h μg ml^{-1} (± S.E.))
TOB-SLN	36.30* ± 1.09	4.0	155.08[b] ± 4.31
Tobral®	20.01 ± 0.48	0.5	37.19 ± 1.14

[a] Calculated from t = 0 to 360 min for TOB-SLN, and from $t = 0$ to 240 min for Tobral®.
[b] Significantly different from the reference solution ($P < 0.05$).

The SLN dispersions were perfectly tolerated: in no case the relevant test evidenced symptoms of ocular irritation.

The aqueous humour TOB concentration vs. time profiles resulting from administration to rabbits of the formulations under study are illustrated in Fig. 1; the data are reported as means ± S.E. ($n = 6$). The statistical significance of the differences between mean concentration values in aqueous humour was evaluated using an ANOVA test (StatView Software, Abacus Concepts Inc., Berkeley, CA, USA). The evaluation included calculation of means and standard errors (S.E.) and group comparison using the Fisher PLSD test. Differences were considered significant at $P < 0.05$. As shown in the figure, significantly higher TOB aqueous humour levels were provided by TOB-SLN with respect to Tobral® from one hour up to six hours after instillation. The tests were discontinued after six hours, since it was considered that sufficient evidence had been gathered, in this preliminary study, on the superiority of the SLN formulation with respect to the reference one.

The pharmacokinetic parameters for TOB in the aqueous humour are summarised in Table 3. The AUC values (areas under the concentration vs. time curves) for TOB in aqueous humour were obtained from the curves in Fig. 1 using the linear trapezoidal rule (Kaleidagraph, Synergy Software). The data are clearly indicative of the greater TOB bioavailability produced by the

TOB-loaded SLN, when compared with the reference eyedrops. The former vehicle produced a C_{max} increase (1.5-fold), a t_{max} increase (8-fold), and a 4-fold AUC increase with respect to the reference vehicle. The increased TOB bioavailability produced by SLN is possibly to be attributed to their longer preocular retention time with respect to the reference solution, as evidenced by the retention study SLN, due to their extremely small size, are presumably entrapped and retained in the mucin layer covering the corneal epithelium. Another hypothesis, to be further verified, is the enhancement of corneal penetration of the drug caused by Epikuron 200 (soya phosphatidylcholine, a surfactant) present on the SLN surface.

In conclusion, SLN appear as a promising vehicle for topical ocular administration of tobramycin. Their use might replace with advantage subconjunctival injections, which are necessary for treatment of 'resistant' pseudomonal keratitis, or for prophylaxis against bacterial endophthalmitis, before cataract surgery (Furgiuele et al., 1978; Ellis and Riegel, 1988).

References

Assil, K.K., Frucht-Perry, J., Ziegler, E., Schanzlin, D.J., Schneiderman, T., Weinreb, R.N., 1991. Tobramycin liposomes. Single subconjunctival therapy of pseudomonal keratitis. Invest. Ophthalmol. Vis. Sci. 32, 3216–3220.

Bargoni, A., Cavalli, R., Zara, G.P., Fundarò, A., Caputo, O., Gasco, M.R., 2001. Transmucosal transport of tobramycin incorporated in solid lipid nanoparticles (SLN) after duodenal administration to rats. Part II—Tissue distribution. Pharmacol. Res. 43, 497–502.

Bottari, F., Giannaccini, B., Cristofori, B., Saettone, M.F., Tellini, N., 1978. Semisolid ophthalmic vehicles I. A study of eye irritation in albino rabbits of a series of gel type aqueous bases. Il Farmaco Ed. Pratica 10, 434–446.

Cavalli, R., Morel, S., Gasco, M.R., Saettone, M.F., Chetoni, P., 1995. Preparation and evaluation in vitro of colloidal lipospheres containing pilocarpine as ion-pair. Int. J. Pharm. 117, 243–246.

Cavalli, R., Gallarate, M., Gasco, M.R., 1999. Incorporation of tobramycin into solid lipid nanospheres as ion-pairs complexes. Acta Technol. Legis. Medic. 10, 17–27.

Cavalli, R., Zara, G.P., Caputo, O., Bargoni, A., Fundarò, A., Gasco, M.R., 2000. Transmucosal transport of tobramycin incorporated in SLN after duodenal administration to rats. Part I—a pharmacokinetic study. Pharmacol. Res. 42, 541–545.

Domb, A.J., 1995. Long-acting oxytetracycline Formulations. Int. J. Pharm. 124, 271–278.

Ellis, P.P., Riegel, M., 1988. Prolonged aqueous humor levels of subconjunctival antibiotics after treatment with acetazolamide and/or timolol. Ophthalmic Surg. 19, 501–505.

Frucht-Perry, J., Assil, K.K., Ziegler, E., Douglas, H., Brown, S.I., Schanzlin, D.J., Weinreb, R.N., 1992. Fibrin-enmeshed tobramycin liposomes: single application topical therapy of Pseudomonas keratitis. Cornea 11, 393–397.

Furgiuele, F.P., Smith, J.P., Baron, J.G., 1978. Tobramycin levels in human eyes. Am. J. Ophthalmol. 85, 121–123.

Gasco, M.R., 1997. Solid lipid nanospheres from warm microemulsions. Pharm Technol. Eur. 9, 52–58.

Gasco, M.R., 2001. Solid lipid nanoparticles for drug delivery. Pharm Technol. Eur. 13, 32–42.

Giunchedi, P., Conte, U., Chetoni, P., Saettone, M.F., 1999. Pectin microspheres as ophthalmic carriers for piroxicam: evaluation in vitro and in vivo in albino rabbits. Eur. J. Pharm. Sci. 9, 1–7.

Müller, R.H., Mader, K., Gohla, S., 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art. Eur. J. Pharm. Biopharm. 50, 161–177.

Saettone, M.F., Giannaccini, B., Chetoni, P., Torracca, M.T., Monti, D., 1991. Evaluation of high- and low-molecular weight fractions of sodium hyaluronate and an ionic complex as adjuvants for topical ophthalmic vehicles containing pilocarpine. Int. J. Pharm. 72, 131–139.

Siekman, B., Westesen, K., 1992. Submicronized parenteral carrier systems based on solid lipid nanoparticles. Pharm. Pharmacol. Lett. 1, 123–126.

Walker, P.E., Coates, P.E., 1981. High performance liquid chromatographic method for determination of gentamicin in biological fluids. J. Chromat. 223, 131–138.

Yang, S., Zhu, J., Lu, Y., Liang, B., Yang, C., 1999. Body distribution of camptothecin solid lipid nanoparticles after oral administration. Pharm. Res. 16, 751–757.