Poly(ε-Caprolactone) Nanocapsules in Carteolol Ophthalmic Delivery

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In order to increase the ocular absorption of carteolol, this antiglaucomatous drug was incorporated into either nanoparticles (NP) or nanocapsules (NC). The polymer used was poly(ε-caprolactone) (PCL). The dosage forms were tested on intraocular hypertensive-induced rabbits. Results are presented as the chronological variations of the intraocular pressure (IOP) in comparison with the commercial aqueous solution (Carteol eye drops). The therapeutic results (decrease in IOP) were much more pronounced with carteolol incorporated into the colloidal carriers than with the commercial eye drops. Further, NC displayed a better effect than NP because the drug was entrapped in the oily core of the carrier, thus more readily available to the eye. The incorporation of the drug into nanocapsules produced a decline in the cardiovascular side effects in comparison with aqueous eye drops, thus showing that the undesired noncorneal absorption was reduced. In conclusion, colloidal suspension made of poly(ε-caprolactone) could offer a good opportunity for ophthalmic delivery of drugs.

KEY WORDS: nanoparticles; nanocapsules; intraocular pressure; glaucoma; poly(ε-caprolactone); carteolol.

INTRODUCTION

Eye drops are generally presented as aqueous solutions. Due to the tear turnover, the lachrymal drainage and the hydrophobic structure of the corneal epithelium, the ocular bioavailability of a majority of drugs is less than 10% (1–3). Moreover, the proportion of drug which does not diffuse through the cornea (about 90% of the instilled drug amount) may be absorbed directly in the systemic circulation by the conjunctival and nasal blood vessels, thereby exerting secondary effects (4).

Therefore, the objectives of this work were to prepare a new ocular drug delivery system which is well tolerated and not only will prolong the precorneal residence time of drugs but also can deliver the drug in its nonionized form: theoretically the lipophilic drug should be absorbed at a greater rate by epithelial cells (5), where it could be transformed in the ionized form by physiological buffers. Under these conditions, the drug could diffuse rapidly through the cornea and the systemic absorption could be reduced.

Two different polymeric colloidal carriers were prepared: nanoparticles (matrix structure) and nanocapsules (vesicular structure) which are made of an oily core surrounded by a thin polymeric wall. Due to previous results, poly(ε-caprolactone) (PCL) was chosen as the polymer (6).

The hydrophilic nonselective β-blocking agent carteolol, used worldwide as an antiglaucomatous drug, was chosen as the drug model. The drug has been either linked to the surface of the nanoparticles by an adsorption process or incorporated into the nanoparticles and the nanocapsules. The therapeutic effect of carteolol, i.e., the decrease in intraocular pressure (IOP), was studied in ocular hypertensive rabbits in comparison with commercial eye drops (Carteol, Chauvin-Blache Laboratories, Montpellier, France). The secondary cardiovascular side effects of the drug encapsulated into nanocapsules were also evaluated in rabbits and compared with the effects induced by carteolol solutions.

MATERIALS AND METHODS

Chemical Agents

PCL (Mw 42,000) was supplied by Aldrich-Chemie (Steinheim, Germany). Carteolol chlorhydrate (batch No. 5I71) was donated by Chauvin-Blache Laboratories. TiO_5 is made up of free and esterified fatty acids (in particular, linoleic and linolenic) and was kindly furnished by Henkel (Dusseldorf, Germany). Pluronic F68 was provided by BASF (Paris, France). All reagents used were analytical grade.

Preparation of Nanoparticles

PCL nanoparticles were prepared as described by Fessi *et al.* (7). Briefly, 0.125 g of polymer was dissolved in 20 mL of an acetonic phase. This phase was added to 50 mL of an aqueous solution containing 0.25 g of Pluronic F68 as a stabilizer. Acetone was eliminated and the final volume of the suspension was adjusted to 5 mL by evaporation under reduced pressure. The adsorption of carteolol under the chlorhydrate form was obtained by adding the drug to the preformed nanoparticle suspension, while stirring it for 1 hr. The incorporation of carteolol chlorhydrate into the carriers was achieved by dissolving the drug in the aqueous medium before the addition of the polymer in the organic phase. The nonionized form was incorporated by previously dissolving the drug under the base form in the acetonic phase. The final drug concentration of carteolol (expressed under the base form) was 1%.

Preparation of Nanocapsules

The preparation method (7) is similar to the nanoparticle method of preparation. However, drugs can be incorporated only into these vesicular structures. Carteolol in the nonionized form was first dissolved in 0.5 mL of the oily phase (TiO5): this phase was further dissolved with the polymer in the acetonic phase. The final volume of the suspensions was also adjusted to 5 mL. Based on preliminary results, the final concentration of carteolol was adjusted to 0.2%.

Comparison with Commercial Eye Drops

In the case of Carteol (1% of carteolol chlorhydrate, pH

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7.4), the formulation includes 0.005% benzalkonium chloride. Since benzalkonium chloride is known to increase ocular absorption, the same concentration of preservative agent as in the commercial eye drops was added to the different colloidal carriers preparations. The pH was also adjusted to 7.4 with 0.1 *M* HCl.

Physicochemical Characterization of the Suspensions

Diameter and Zeta Potential. Particle and capsule diameters were determined by photonic correlation spectroscopy (Malvern 4600, Malvern, England), and zeta potentials by laser Doppler velocimetry (Zetasizer II, Malvern, England). All preparations were diluted with a 10^{-6} *M* NaCl solution in order to maintain a constant ionic strength and an adequate concentration of drug carriers.

Determination of Entrapment Efficiency. The nonentrapped drug was separated from the carriers by ultracentrifugation (20,000*g*, 90 min). The supernatant (in the case of NP) or the infranant (in the case of NC) containing free-drug was removed and the drug concentration measured by HPLC. The amount of entrapped drug was calculated according to the following equation:

$$\text{(entrapped drug)} = \text{(total drug)} - \text{(nonentrapped drug)}$$

In Vivo Experiments

Experimental Induction of Ocular Hypertension. The method of induction of ocular hypertension is derived from Vareilles and co-workers' experiments (8). Rabbits were first anesthetized with an injection of Nembutal, 5% (0.4 mL/kg), in the marginal ear vein, then 200 μL of α-chymotrypsin (500 U/mL) was injected in the posterior chamber of the eye. Approximately 4 months later, the IOP was found to be stable and its value varied between 30 and 50 mm Hg.

Tests of Reduction of the IOP. Twenty-five microliters of the tested suspension was administered in the cul-de-sac of nine rabbits. The evolution of the IOP was directly measured for 8 hr with an aplanation tonometer (Alcon). After a 48-hr washout period, 25 μL of the commercial eye drops (Carteol) was administered in the same eye, thus serving as control eye drops. Statistical analysis was performed using a one-factor analysis of variance followed by a Fischer test.

Evaluation of Cardiac Side Effects. Rabbits were anesthetized with pentobarbital (5 mg/kg) via the marginal ear vein. The femoral artery was cannulated and heart rate and blood pressures were recorded using a ratemeter (Model 7302, Narco Biosystems) driven by the pulse pressure signal. In order to determine whether the instillation of a β-blocking agent in the cul-de-sac induces a blockage of the cardiovascular β-adrenergic system, the ability of a β-adrenergic agonist (isoprenaline) to induce an increase in heart rate and a decrease in blood pressures after injection in the marginal ear vein was studied (3).

First, an injection of isoprenaline (0.5 mg/kg) was performed. A higher heart rate and lower blood pressures were registered. When these values returned to the baseline, 25 μL of eye drops was instilled in the cul-de-sac of one eye. Sixty minutes later a new injection of isoprenaline (same concentration as before) was performed and the cardiovascular response was registered.

Each dosage form was tested on three rabbits. This experiment was carried out only with the colloidal suspension displaying the best therapeutic effect, i.e., nanocapsules with a concentration of carteolol of 0.2%. Carteol, 1%, and a carteolol chlorhydrate solution (0.2%) were chosen as references.

RESULTS

Physicochemical Characteristics

Average diameters and zeta potentials are listed in Table I. It appears that the diameters of the dispersed particles average 150 nm. Nanocapsules are larger than nanoparticles and their diameters are about 300 nm. These sizes are small enough to avoid discomfort after instillation.

The zeta potentials of both nanoparticles and nanocapsules are negative. The surface charge of nanoparticles is poorly affected by the adsorption of carteolol.

Depending both on the type of carrier (NP or NC) and on the physicochemical state of the drug (ionized or nonionized), the percentages of drug associated with the carriers may be different (Table I). Carteolol chlorhydrate is not adsorbed onto nanoparticles and the incorporation percentages of carteolol in the nonionized form into nanoparticles and nanocapsules are about 40 and 66%, respectively.

Reduction in IOP

The reductions in IOP induced by the instillation of 25 μL of nanoparticle suspension are presented in Figs. 1 and 2. It appears that when the drug is incorporated as the nonionized form into nanoparticles, the reduction in IOP is more pronounced than when it is adsorbed or incorporated as the chlorhydrate form (Fig. 1).

Figure 2 shows that the presentation of the drug in its nonionized form into nanoparticles increased the therapeutic effect compared with the commercial solution eye drops.

Table I. Physicochemical Characteristics of Polymeric Drug Carriers

	NP	NC
Diameters (nm)		
Without drug	139 (0,1)[a]	273 (0,2)
Carteolol Cl^- adsorbed	134 (0,1)	ND
Carteolol Cl^- incorporated	159 (0,2)	ND
Carteolol base incorporated	175 (0,2)	304 (0,2)
Zeta potentials (mV)		
Without drug	−33	−36
Carteolol Cl^- adsorbed	−32	ND
Carteolol Cl^- incorporated	−31	ND
Carteolol base incorporated	−32	−34
Adsorption ratio (%)		
Carteolol chlorhydrate, 1%	<2	ND
Incorporation ratio (%)		
Carteolol chlorhydrate, 1%	39	ND
Carteolol base, 0.2%	44	ND
Carteolol base, 0.2%	ND	66

[a] Polydispersity index in parentheses.

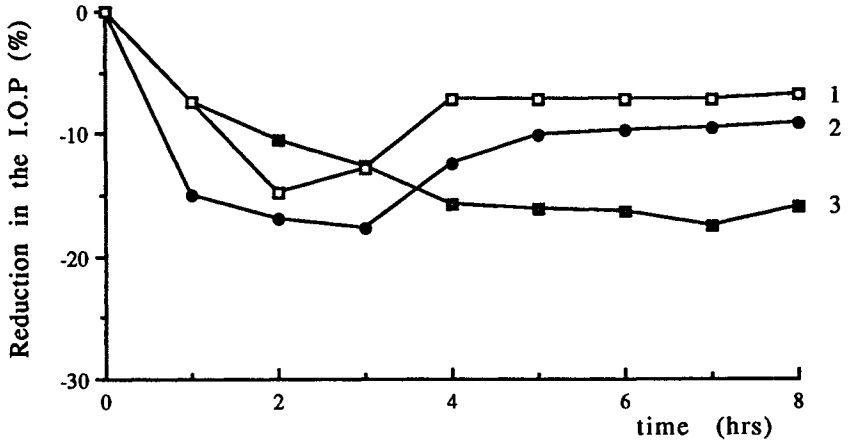

Fig. 1. Chronological evolution of the IOP after instillation of carteolol chlorhydrate incorporated into nanoparticles (1) or adsorbed onto nanoparticles (2) or Carteol (3). The concentration of carteolol chlorhydrate is 1% in each preparation.

Indeed, a 0.2% concentration of carteolol induces a response equivalent to the commercial solution (1%) when the drug is incorporated into PCL nanoparticles. Moreover, when carteolol is encapsulated into nanocapsules at a concentration as low as 0.2%, it induces a higher and a more prolonged reduction in IOP than Carteol, which contains five times the amount of drug (1%).

Cardiovascular Side Effects

Figure 3 confirms that isoprenaline injection induces an increase in heart rate and a decrease in systolic and diastolic blood pressures in nontreated rabbits. It clearly appears that the instillation of 25 μL of Carteol inhibits the increase in heart rate and the lowering of blood pressures for at least 60 min following an isoprenaline injection. The same effect is observed after instillation of a 0.2% carteolol solution. In contrast, there is no significant difference in the increase in heart rate when isoprenaline is injected before or 60 min after instillation of nanocapsules containing carteolol. Moreover, after instillation of encapsulated carteolol, the blood pressures are partially reduced by isoprenaline injection.

DISCUSSION

We have previously demonstrated that nanoparticles made of either a copolymer of lactic and glycolic acid (PLAGA) or poly(isobutylcyanocarylate) (PIBCA) is unable to increase the pharmacological effect of betaxolol (6,9). As PLAGA and PIBCA carriers are rather hydrophilic, we studied another polymer, PCL, which is a more hydrophobic polymer.

Carteolol chlorhydrate is not adsorbed onto PCL nanoparticles (Table I): under these conditions, the drug is administered as a free-drug solution and PCL nanoparticles do not increase the reduction in the IOP in comparison with the commercial aqueous solution (Fig. 1).

In order to associate the hydrophilic drug with nanoparticles more strongly, one possibility would be to incorporate the drug into the nanoparticles during their preparation process. In this case, the percentage of carteolol chlorhydrate associated with nanoparticles increases to 41%. However, Fig. 1 shows that the reduction in IOP induced by carteolol chlorhydrate incorporated into nanoparticles is less marked than when the drug is presented as the aqueous solution. This result confirms that the incorporation of a drug into

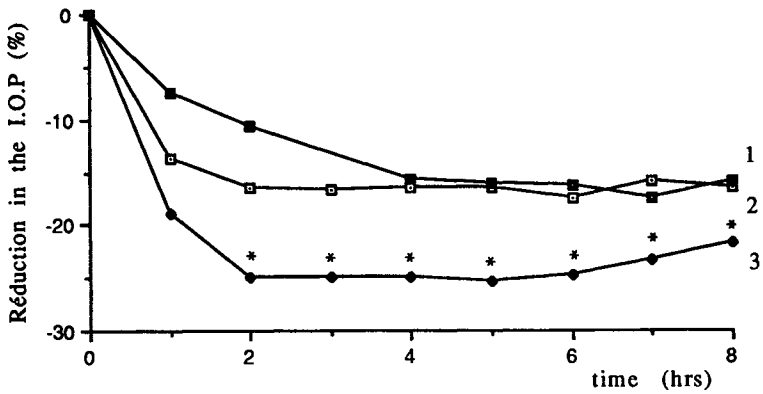

Fig. 2. Chronological evolution of the IOP after instillation of Carteol (1) or 0.2% nonionized carteolol incorporated into nanoparticles (2) or incorporated into nanocapsules (3). (*) Statistically different from 1 and 2.

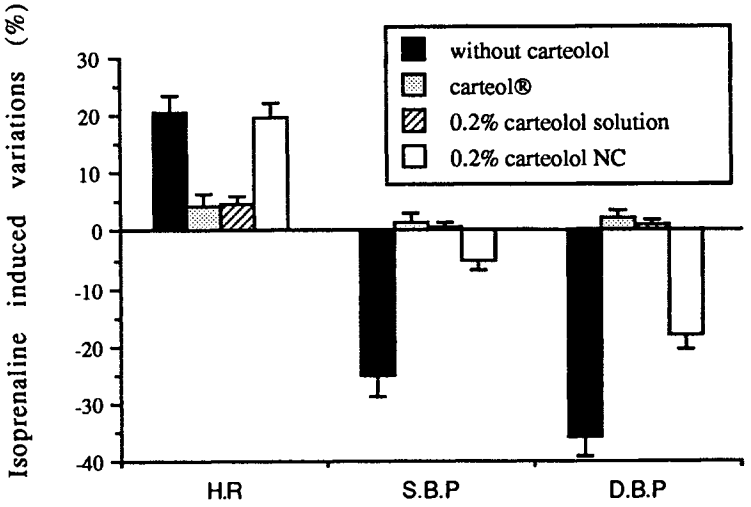

Fig. 3. Heart rate (H.R), systolic blood pressure (S.B.P), and diastolic blood pressure (D.B.P) responses to intravenous isoprenaline injection before and 60 min after instillation of 25 μL of the different dosage forms.

nanoparticles reduced the transcorneal penetration of the drug as shown by Harmia *et al.* in the case of pilocarpine and PIBCA (10).

With a view to enhancing the ocular activity, the physicochemical nature of this β-blocking agent was modified. Indeed, carteolol was incorporated into PCL carriers in the nonionized form. Figure 2 shows that the reduction in IOP is much more pronounced when the drug is incorporated into nanoparticles or nanocapsules in the nonionized form than when it is in the chlorhydrate solution. Two major mechanisms can be involved in this improvement. First, the corneal epithelium is a hydrophobic tissue which is more permeable to lipophilic species such as the nonionized form of drugs. In addition, diffusion of the drug through the hydrophilic stroma is not restricted since the nonionized drug may be converted into its ionized form by the physiological buffers. *In vitro* results confirming our experiments were recently published (11): this work showed that raising the pH of ophthalmic formulations from 7.4 to 8.4 induces an increase in the ratio corneal/conjunctival penetration of some β-blockers partly by increasing the fraction of the nonionized drug. Second, the behavior of PCL carriers after instillation in the cul-de-sac is important. Indeed, PCL nanoparticles undergo an agglomeration process after instillation, thus ensuring a prolonged residence time of the drug in front of the eye. This behavior was not observed with PLAGA or PIBCA polymers (6). One hypothesis which could explain this result is that the carrier is hydrophobic enough to interact with proteins of the lachrymal fluid as already observed in the case of i.v. administration of colloidal carriers, where opsonization is more important for hydrophobic structures (12). Consequently, the hydrophobic interactions could be more important than the electrostatic ones: the repulsive electrostatic forces created between the negatively charged corneal epithelium and the negatively charged particles (Table I) could be inefficient with regard to the agglomeration process.

Two different colloidal carriers may be used to incorporate drugs in their nonionized form, i.e., nanoparticles or nanocapsules. The comparison of the two colloidal carriers clearly shows that the transcorneal penetration of carteolol is much more pronounced when the drug is incorporated into nanocapsules rather than into nanoparticles (Fig. 2). Moreover, the instillation of nanocapsules with a carteolol concentration of 0.2% induces a better reduction in the IOP in intensity and in time than the 1% carteolol chlorhydrate solution (Carteol). The advantage of nanocapsules seems to be due to the presence of an oily core. Indeed, this vesicular structure may incorporate higher amounts of lipophilic drugs (66%) than the matrix structure of NP (40%) (13). In addition, compared to nanoparticles, better protection against the transformation into the ionized form by the lachrymal fluid can be offered by the oily core of nanocapsules. Another possibility is that diffusion of the drug from the oily phase toward the epithelium may be more effective than diffusion from the internal matrix of the particles.

Furthermore, when carteolol is incorporated in nanocapsules, the increase in the therapeutic effect allows a dramatic reduction in cardiovascular side effects. The inability of isoprenaline to increase the heart rate after instillation of either a 1 or a 0.2% carteolol solution (Fig. 3) demonstrates that the ocular administration of this drug blocks the β1-adrenergic receptors of the heart. The β2 receptors of blood vessels are also blocked for at least 60 min after the instillation of each carteolol solution. In contrast, β1 and β2 receptors react to isoprenaline injection after the instillation of encapsulated carteolol, thus showing a dramatic change compared to the isoprenaline response after administration of carteolol solutions. On the basis of such evidence, one can conclude that the incorporation of nonionized drug into PCL nanocapsules decreases the overall systemic absorption of drug after ocular administration. Consequently, the ratio corneal/conjunctival penetration of this drug could be increased, thus explaining the reduction of the systemic side effects.

In conclusion, after ocular administration, the ability of PCL colloidal carriers to increase the therapeutic effect of carteolol, a nonselective hydrophilic β-blocking agent, has

been demonstrated. PCL colloidal carriers display two major characteristics: they can extend the residence time of the drug in front of the cornea as well as make it possible to deliver the drug in its unionized form, which is more favorable for corneal absorption. In addition, the incorporation of the drug into PCL nanocapsules greatly reduces its absorption by the systemic circulation, thus reducing its β-adrenergic cardiovascular blocking activity.

Colloidal suspensions made of PCL could offer a good opportunity for ophthalmic delivery of lipophilic, hydrophilic (after transformation in the nonionized form), insoluble drugs and drugs with severe secondary effects. Furthermore, PCL has already been administered subcutaneously to man, which demonstrates the potential of this polymer for medical use (14). It should also be noted that the process at the origin of the carriers' agglomeration is now under study.

REFERENCES

1. V. H. L. Lee and J. R. Robinson. Review: Topical ocular drug delivery. Recent developments and future challenges. *J. Ocul. Pharm.* 2:67–108 (1986).
2. S. C. Chang and V. H. L. Lee. Nasal and conjunctival contributions to the systemic absorption of topical timolol in the pigmented rabbit: Implications in the design and strategies to maximize the ratio of ocular to systemic absorption. *J. Ocul. Pharm.* 3:159–169 (1987).
3. D. Sirbat, C. Kholer, J. L. George, D. Mayeux, and J. P. Grilliat. Evaluation des effets systémiques cardiovasculaires et bronchiques d'un collyre β-bloquant. *Ophtalmologie* 2:213–218 (1988).
4. S. Ball. Congestive heart failure from betaxolol. *Arch. Ophthalmol.* **105**:320 (1987).
5. K. Kishida and T. Otori. A quantitative study on the relationship between transcorneal permeability of drugs and their hydrophobicity. *Jpn. J. Ophthalmol.* 24:251–259 (1980).
6. L. Marchal-Heussler, H. Fessi, J. P. Devissaguet, M. Hoffman, and Ph. Maincent. Colloidal drug delivery systems for the eye. A comparison of the efficacy of three different polymers: Polyisobutylcyanoacrylate, polylactic-co-glycolic acid, polyepsilon-caprolactone. *STP Pharma Sci.* 2:98–104 (1992).
7. H. Fessi, F. Puisieux, J. P. Devissaguet, N. Ammoury, and S. Benita. Nanocapsules formation by interfacial polymer deposition following solvent displacement. *Int. J. Pharm.* 55:R1–R4 (1989).
8. P. Vareilles, P. Conquet, and J. C. Le Douarec. A method for the routine intraocular pressure (IOP) measurement in the rabbit: Range of IOP variations in this species. *Exp. Eye Res.* 24:269–282 (1977).
9. L. Marchal-Heussler, Ph. Maincent, M. Hoffman, J. Spittler, and P. Couvreur. Antiglaucomatous activity of betaxolol chlorhydrate sorbed onto different isobutylcyanoacrylate nanoparticle preparations. *Int. J. Pharm.* 58:115–122 (1990).
10. T. Harmia, J. Kreuter, P. Speiser, T. Boye, R. Gurny, and A. Kubis. Enhancement of the myotic response of rabbits with pilocarpine-loaded polybutylcyanoacrylate nanoparticles. *Int. J. Pharm.* 33:187–193 (1986).
11. P. Ashton, S. K. Podder, and V. H. L. Lee. Formulation influence on conjunctival penetration of four beta blockers in the pigmented rabbit: A comparison with corneal penetration. *Pharm. Res.* 9:1166–1174 (1991).
12. S. D. Troster, U. Muller, and J. Kreuter. Modification of the body distribution of poly(methylmethacrylate) nanoparticles in rats by coating with surfactants. *Int. J. Pharm.* 61:85–100 (1990).
13. N. Ammoury, H. Fessi, J. P. Devissaguet, F. Puisieux, and S. Benita. Physicochemical characterization of polymeric nanocapsules and in vitro release evaluation of indomethacin as a drug model. *STP Pharma* 5:647–651 (1989).
14. S. J. Ory, C. B. Hammond, S. G. Yancy, R. W. Hendren, and C. G. Pitt. The effect of a biodegradable contraceptive capsule (Capronor) containing levonorgestrel on gonadotropin, estrogen, and progesterone levels. *Am. J. Obstet. Gynecol.* 5:600–605 (1983).