International Journal of Pharmaceutics, 58 (1990) 115–122
Elsevier

IJP 01963

Antiglaucomatous activity of betaxolol chlorhydrate sorbed onto different isobutylcyanoacrylate nanoparticle preparations

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(Received 3 March 1989)
(Accepted 1 July 1989)

Key words: Isobutylcyanoacrylate nanoparticle; Betaxolol chlorhydrate; Glaucoma; Ophthalmic drug delivery system; Glaucomatous rabbit

Summary

The objectives of the present work were to evaluate the effects, on the reduction of the intra-ocular pressure (IOP) in glaucomatous rabbits, of different physico-chemical parameters such as: (1) the adsorption percentage of an antiglaucomatous drug (betaxolol chlorhydrate) onto isobutylcyanoacrylate nanoparticles; (2) the binding type of the drug onto the surface of the particles (hydrophobic or electrostatic). By modification of the surface charge, it was possible to adsorb either 25, 30 or 70% for the same drug concentration. When administered in glaucomatous rabbits, in comparison with commercial eye drops, the suspension with the highest adsorption level considerably lowered the overall therapeutic activity. The suspension with the lowest drug payload did not significantly increase the maximal intensity of the therapeutic response and tended to prolong the reduction of the IOP in time. It has been clearly shown that, regarding the ocular administration, the surface charge of the particles and the binding type of the drug onto the nanoparticles were much more important parameters than the drug adsorption percentage onto the nanoparticles.

Introduction

Since the development of alkylcyanoacrylate nanoparticles by Couvreur et al. (1982), many articles have been published dealing mostly with their enhancement of therapeutic efficacy, especially with anticancer drugs (Grislain et al., 1983; Kreuter and Hartmann, 1983; Illum et al., 1984). Most of the time, the colloidal carriers were administered by intravenous route. More recently, Maincent et al. (1986) administered vincamine adsorbed onto nanoparticles to rabbits by the oral route and these authors showed an important improvement of the bioavailability of the drug.

Due to their adhesives properties (Refojo et al., 1971), alkycyanoacrylate nanoparticles could also provide interesting effects in the area of ophthalmology. So Wood et al. (1985) have shown that hexylcyanoacrylate nanoparticles remained longer in the tear film than ophthalmic solutions; these results have been confirmed by gamma-scintigraphic studies with polybutylcyanoacrylate nanoparticles (Fitzgerald et al., 1987).

From these observations, it seemed probable that the alkylcyanoacrylate colloidal suspension could increase the residence time of drugs in the

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Part of this work has been presented at the 15th Controlled Release Society Meeting, August 1988, Basel, Switzerland.

tear film and therefore prolong the penetration of drugs into the intraocular structures.

Drugs can be either incorporated in the matrix of the polymer particle or adsorbed onto the surface of the colloidal carrier, depending on the addition of the drug either before or after the polymerisation process. For ophthalmic purposes, Harmia et al. (1986) have demonstrated that the linkage of pilocarpine chlorhydrate onto butylcyanoacrylate nanoparticles by a sorption process induced a longer myosis compared to the incorporation of the drug into the particles: all their experiments with pilocarpine were carried out with particle adsorbing the same amount of drug (15% of the drug amount) (Kreuter et al., 1988).

The aim of the present paper was to demonstrate that by modifying the particles' surface characteristics, it may be possible to obtain different drug payloads onto the particles for a same initial concentration of the drug in the suspension, since this parameter is likely to be a very important one to determine the ability of the carrier to prolong the acting time of the drug. In addition, the therapeutic activity of the prepared suspensions was studied. Betaxolol chlorhydrate, a β_1-blocking agent, used for its antiglaucomatous activity (Betoptic ®, Alcon Laboratories) was chosen as a drug model. Thus, the therapeutic activity has been measured owing to the reduction of the intraocular pressure (IOP) in glaucomatous rabbits.

Materials and Methods

Materials

Isobutylcyanoacrylate (IBCA) (Ethnor, Paris) was used for the monomer. Betaxolol chlorhydrate (batch No. MP114, Alcon, Kaysersberg, France) was the drug model. Dextran 70000, dextran sulfate and *N*-acetylglucosamine (Sigma, St. Louis, MO) were used as suspension stabilisers. All other reagents were of analytical grade.

Preparation of isobutylcyanoacrylate (IBCA) nanoparticles

Nanoparticles were synthetized following a previously published method (Couvreur et al., 1982). Briefly, 1 g IBCA was added drop by drop to 100 ml of an acidic aqueous solution (10^{-3} M HCl) containing three different stabilisers: that is, first dextran 70000 (1%) in suspension A, second a mixture of dextran 70000 (0.8%) and dextran sulfate (0.3%) in suspension B, third a mixture of dextran 70000 (0.5%) and *N*-acetylglucosamine (0.5%) in suspension C. Then the suspensions were stirred for 2 h at room temperature with a magnetic stirrer. The resulting suspensions were filtered through a glass filter and neutralized with 0.1 N NaOH to pH 7.4.

Physico-chemical characteristics of the particles

The size of the particles without and with betaxolol chlorhydrate was determined by LASER diffraction method (Malvern 4600, U.K.).

Zeta-potentials were determined by means of an electrophoresis cell: in this system, the particles (pH 7.4, 25 °C) were placed in a U-cell and a potential of − 80 mV was established. The motion of the particles in this electric field was observed with a camera and the speed of their motion was measured. Finally, the zeta-potential was calculated with the Schmoluckovsky formula.

Adsorption isotherms

An appropriate amount of betaxolol chlorhydrate (0.26, 0.36, 0.46, 0.56, 0.66, 0.86%, w/v) was added to 10 ml of suspension A, B and C, and magnetically stirred for 1 h at room temperature.

In order to determine the adsorbed amount of betaxolol chlorhydrate onto the nanoparticles, 5 ml of the resulting suspension were centrifuged (27000 rpm. 90 min) (L5 50, Beckman) and the betaxolol chlorhydrate remaining in the supernatant was then analysed by HPLC (ALCON, unpublished method). Briefly, a Spectra-Physics liquid chromatograph system (SP8700 solvent delivery system, SP8750 organiser, SP8400 detector, SP4200 integrator) fitted with a Waters column (C18, 5 μm, 12 cm) were used. A 20-μl sample loop was used to inject the sample into the analytical column. The diluted samples of supernatant (dilution 1/400) were chromatographed with a mobile phase constituted by phosphate buffer, acetonitrile and dimethylamine chlorhydrate (65;35;0.5, v/v/w). The flow rate was 1.5

ml/min and the detector wavelength was 220 nm. Standard solutions of betaxolol chlorhydrate were chromatographed before and after the experimental samples, which allowed to plot the reference line.

In vitro drug release

The in vitro desorption of betaxolol chlorhydrate from nanoparticles was studied by means of a dialysis technique (Gupta et al., 1987). Suspensions A–C containing 0.56% (w/v) betaxolol chlorhydrate as for the commercial eye drops concentration, were tested. In order to take into account the very low dilution of the drug in the eye, 4 ml of the suspensions were diluted by 1 ml of phosphate buffer (pH 7.4) and placed inside the cellulose dialysis tubing tied at one end (molecular weight cut-off 8000, Polylabo, Strasbourg). The suspensions were dialysed at 37 °C against 200 ml of purified water (sink conditions); 500-μl samples of the dialysis medium were taken out at the following times: 5, 10, 15, 20, 25, 30, 40, 50, 60, 90 min. No replacement of the dialysis medium was performed, but the final results were corrected according to the variation of the dialysis medium bulk. The same experiments were made with betaxolol chlorhydrate solutions in phosphate buffer (pH 7.4). In order to display the desorption process of the drug from the particles, the betaxolol chlorhydrate concentration in each dialysis solution was equal to the concentration of the free drug in the three different nanoparticulate suspensions, i.e. 70% for solution A, 35% for solution B, 75% for solution C. Consequently, the differences between the profiles of the dialysis resulting curves of the nanoparticles and the betaxolol chlorhydrate solutions could only result from the desorption of the drug from the particles.

The amounts of betaxolol chlorhydrate dialysed were determined by the above described HPLC method.

Cation exchange ability of the particles

The cation exchange ability of the particles was examined by monitoring the desorption of the drug upon addition of calcium chloride ($CaCl_2$); a set amount of drug was adsorbed onto the particles (initial concentration of betaxolol chlorhydrate 0.56% i.e. $1.6 \cdot 10^{-4}$ mol/l) and an equivalent amount of $CaCl_2$ (1.6×10^{-4} mol/l i.e., 1.8×10^{-3} %) was incorporated in the three suspensions. After being stirred for 1 h, the suspensions were centrifuged and the amount of betaxolol chlorhydrate remaining in the supernatant was determined.

Experimental induction of glaucoma

The method for the induction of the glaucoma was based on the experiments of Vareilles et al. (1979). After anesthesia of the rabbits by an injection of Nembutal 5% (0.4 ml/kg) into the marginal ear vein, 200 μl of α-chymotrypsin (500 IU/ml) were injected into the posterior chamber of the eye. Approximately 4 months later, the IOP turned out to be stable and its value varied between 30 and 50 mmHg.

Tests of reduction of the IOP

Twenty-five μl of suspension A, B, or C were administered in the cul-de-sac. The evolution of the IOP was directly measured for 7 h with an aplanation tonometer (Alcon). After a 48 h washout, 25 μl of the commercial eye drops (Betoptic®) was administered in the same eye, thus serving as the control eye drop.

Results

Physico-chemical characteristics of the particles

The physico-chemical characteristics of the nanoparticles are presented in Table 1.

All types of free drug nanoparticles have a similar size and a negative zeta-potential. Following the addition of dextran sulfate as an anionic stabilizer in the preparation medium (suspension B), the negative zeta-potential undergoes a 3-fold increase (−45 mV) compared to suspension A which contains only dextran 70000 (−15 mV). The incorporation of *N*-acetylglucosamine in the polymerization medium reduced the zeta-potential (−2 mV).

The adsorption of betaxolol chlorhydrate onto nanoparticles did not alter their size. Nevertheless, the drug adsorption process induced a significant reduction of the zeta-potential of the particles

TABLE 1

Physico-chemical characteristics of the particles at 25 °C and pH 7.4

S.D. = standard deviation; $n = 9$.

	Size (nm) (S.D.)	Zeta-potential (mV) (S.D.)	Adsorption percentage (S.D.)
Suspension A			
without betaxolol chl.	240(3.6)	−15 (1)	
with betaxolol chl.	240(4)	−11 (1.5)	30(3)
Suspension B			
without betaxolol chl.	276(2.8)	−45 (2.5)	
with betaxolol chl.	220(3.1)	−30 (2.1)	65(2)
Suspension C			
without betaxolol chl.	242(3)	−2 (1.5)	
with betaxolol chl.	241(3.8)	+1 (1.5)	22(3)

containing dextran sulfate (−30 mV) but it hardly affected the surface characteristics of suspensions A and C. Although the greatest reduction of the zeta-potential was obtained when the betaxolol chlorhydrate was adsorbed onto the particles containing dextran sulfate (suspension B), the surface of these particles remained the most negative one. The lowest value of the zeta-potential was obtained with suspension C (+1 mV).

Adsorption isotherms

The adsorption isotherms of suspensions A, B and C are shown in Fig. 1. Whatever the stabilizer, the adsorption of betaxolol chlorhydrate was represented by an S-shaped curve: it appeared that

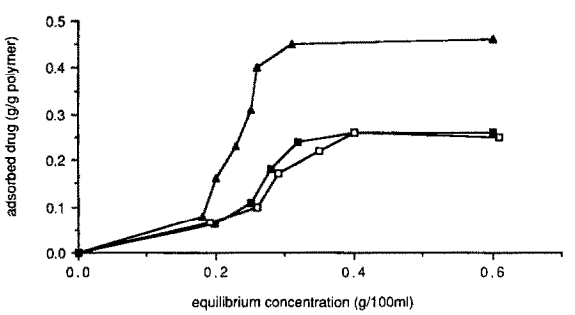

Fig. 1. Adsorption isotherms of betaxolol chlorhydrate. ■, suspension A; ▲, suspension B; □, suspension C.

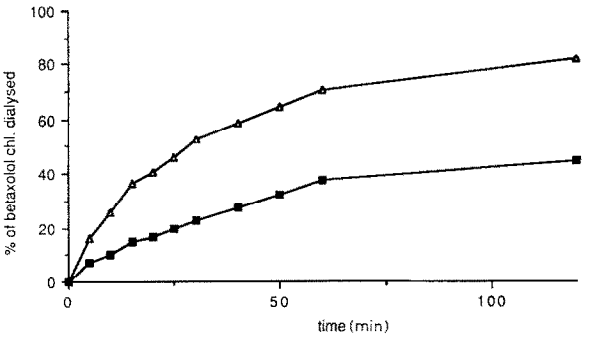

Fig. 2. In vitro dialysis of betaxolol chlorhydrate. ■, suspension A; △, betaxolol solution.

with an equilibrium concentration of 20 mg/10 ml, the adsorption of the drug became easier and a maximum rate of adsorption was reached when the equilibrium concentration was around 0.3 mg/10 ml. The maximal amount of drug adsorbed was 1.5-fold greater with suspension B than with the two others.

In vitro drug release

The in vitro dialysis results of drug release obtained with suspensions A, B and C, in comparison with the dialysis of betaxolol chlorhydrate solutions are presented in Figs. 2, 3 and 4, respectively. In all cases, the profiles of the curves corresponding to the nanoparticles suspensions were significantly different from those obtained with the drug solutions, especially in the first 15 min.

Within this period, the transport of the drug across the dialysis bag was faster with suspension A and C than with the betaxolol chlorhydrate

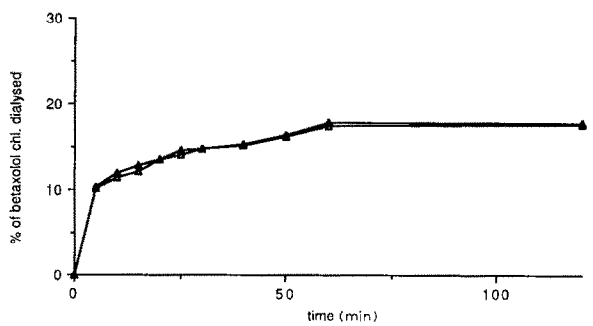

Fig. 3. In vitro dialysis of betaxolol chlorhydrate. ▲, suspension B; △, betaxolol solution.

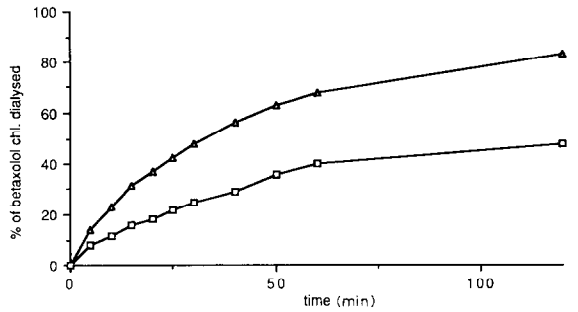

Fig. 4. In vitro dialysis of betaxolol chlorhydrate. □, suspension C; △, betaxolol solution.

solution. Assuming that this transport was supported by Fick's law, the rate of dialysis was directly a function of the difference between the inside and the outside free drug concentrations. Therefore, it appeared that, in the case of suspensions A and C, the inside concentration of free betaxolol chlorhydrate increased rapidly by its desorption from the particles. It is generally believed that the drug release from a carrier such as microspheres follows a zero- or a first-order process. Gupta et al. (1987) proposed the following transformation to study the dialysis process of a drug from a colloidal carrier by plotting:

$$\ln(C_o - C_i V_i/V_t - Q_m/V_t) \text{ vs time}$$

where: C_o is the drug concentration outside the bag, C_i is the drug concentration inside the bag at $t = 0$, Q_m is the total amount of drug at $t = 0$, V_t is the volume of the outside and inside dialysis medium, and V_i is the volume of the inside dissolution medium.

If this transformation of the dialysis results leads to a straight line, it implies that a first-order release rate constant can be determined from the resulting slope. Our dialysis results displayed two different straight lines, allowing the determination of an initial (K_i) and a terminal (K_t) release rate constant. The values of the different release rate constants are summarized in Table 2. Finally, the desorption of betaxolol chlorhydrate was slightly slower in suspension C than in suspension A.

The behavior of suspension B was found completely different. After 480 min of dialysis, only 30% of betaxolol chlorhydrate were seen to be diffused through the bag. This amount corresponded exactly to the amount of non-adsorbed betaxolol chlorhydrate. No major differences could be observed between the curve profiles of nanoparticles and those of betaxolol chlorhydrate solution. Thus, it was clear that no significant desorption of betaxolol chlorhydrate occured for a period of 480 min.

TABLE 2

In vitro release rate constants

	Initial release rate constant (K_i) (g/min per 0.1 g polymer)	Terminal release rate constant (K_t) (g/min per 0.1 g polymer)
Suspension A	0.09	0.01
Suspension B	0	0
Suspension C	0.08	0.02

Cation-exchange ability

The influence of $CaCl_2$ on the binding ability of the particles for betaxolol chlorhydrate is summarized in Table 3. The incorporation of $CaCl_2$ in suspensions A and C poorly affected the adsorption percentage of the drug onto the particles. But such a process induced a dramatic desorption of the drug when the latter was bound to the particles containing dextran sulfate (suspension B).

Tests of reduction of the IOP

The reduction of the IOP obtained after the instillation of suspensions A, B or C and after the instillation of the commercial eye drops are presented in Fig. 5.

TABLE 3

Influence of the incorporation of $CaCl_2$ on the adsorption percentage of betaxolol chlorhydrate

S.D. = standard deviation; $n = 9$.

	Adsorption percentage	
	Without $CaCl_2$ (S.D.)	With $CaCl_2$ (S.D.)
Suspension A	30(2)	31(2.5)
Suspension B	65(1.5)	2(0.8)
Suspension C	22(2.3)	25(2.1)

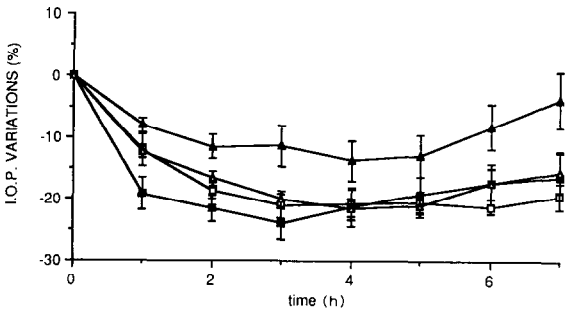

Fig. 5. Percentages of IOP lowering effect vs. time (h) after ocular administration of the different betaxolol preparations. Values are expressed as mean ($n = 10$) ± SE. ■, suspension A; ▲, suspension B; □, suspension C; △, Betoptic®.

Suspension A seemed to induce both a slight increase in the maximal intensity response after 3h and an interesting prolongation of this effect. Suspension C also seemed to prolong the efficacy of the drug but the maximal intensity response appeared to be slightly less important than that of Betoptic®. Actually, the statistical analysis (*t*-test, unpaired series) clearly pointed to the fact that the differences between the therapeutic activity of suspensions A and C on the one hand, and the commercial solution on the other hand were not significant ($p < 0.05$). The therapeutic efficacy of suspension B was found completely different: the nanoparticule dosage form did not prolong the reduction of the IOP while it significantly decreased the maximal intensity response compared to the commercial eye drops ($p < 0.05$).

Discussion

Owing to an increase in the betaxolol chlorhydrate concentration in the colloidal medium, the drug payload onto the nanoparticles is increased up to a maximal amount depending on the particles' surface characteristics.

The adsorption process of betaxolol chlorhydrate onto nanoparticles of suspension A could be assumed as the result of an hydrophobic interaction between the lipophilic part of the drug and the hydrophobic surface of the particles.

In the case of suspension B, following the incorporation of an anionic stabilizer (dextran sulfate) in the particles' matrix, the maximal uptake of betaxolol chlorhydrate was increased to 65% (0.045 g/0.1 g polymer). The hydrophobic part of the dextran sulfate was probably included in or on the polymer while the hydrophilic structure (sulfate anion) appeared at the interface particle–aqueous medium, as already suggested by Douglas et al. (1985). This was confirmed by the increase of the zeta-potential (−15 to −45 mV) which represented the electrophoretic mobility of the particles and clearly showed that the surface of the particles became more negative. When betaxolol chlorhydrate was adsorbed onto the particles, the zeta-potential significantly decreased (−45 to −30 mV). This could be accounted for by the fact that the sulfate anions have become partly masked. At pH 7.4 the amino function of betaxolol chlorhydrate ($pK_a = 9.3$) was present as an ammonium cation. So, it can be assumed that betaxolol chlorhydrate was adsorbed onto the particles essentially because of the interaction due to the sulfate anions of the particles and the ammonium cation of betaxolol chlorhydrate (electrostatic binding). This hypothesis was confirmed by the fact that the drug was rapidly desorbed after addition of $CaCl_2$. In fact, experimental results showed that an amount of Ca^{2+} equivalent to the adsorbed betaxolol chlorhydrate induced a nearly total desorption of the drug. The particles with dextran sulfate act as a cation exchanger.

In the case of suspension C, the incorporation of *N*-acetylglucosamine in the preparation medium also modified the surface of the particles; the zeta-potential was reduced in comparison with the dextran nanoparticles (suspension A). Due to the presence, in the molecule, of an hydrophobic part and a cationic fonction, *N*-acetylglucosamine could be arranged at the surface of the particles in the same way as dextran: thus, the hydrophilic amino group of the molecule was bound at the interface particle–aqueous medium. Yet, the zeta-potential of particles in suspension C was slightly less negative than that of suspension A, which tends to suggest that a small part of *N*-acetylglucosamine was associated to the carrier. The adsorption of betaxolol chlorhydrate onto the particles of suspension C only slightly modifies the zeta-potential; moreover, the maximal adsorption

of betaxolol chlorhydrate was reduced compared to suspension A. This reduction (30–22%) could be due to the presence of some ionized groups of *N*-acetylglucosamine onto the particles surface interacting with betaxolol chlorhydrate by electrostatic repulsions, so that, as for suspension A, only hydrophobic binding was supposed to occur.

These results therefore provided three suspensions containing the same overall concentration of betaxolol chlorhydrate but displaying three distinct rates of adsorption owing to two different binding mechanisms.

The desorption kinetics of the drug from nanoparticles was also influenced by the modification of the surface properties of the particles. This process was represented by an exponential curve for suspension A and C (Figs. 2 and 4, respectively). The exponential curves of the diffusion of the free drug through the dialysis bag showed that this process could obey Fick's law; a significant desorption of the drug from nanoparticles was seen to occur with suspensions A and C. The dialysis results transformation led to a biphasic linear representation (Fig. 6) which showed that the release process followed a double first-order kinetic. Two different rates of desorption were obtained depending on the initial or the terminal part of the curve: the release rate constants were quite similar for suspensions A and C (see Table 2). These relatively important rates of initial desorption confirmed that the major part of betaxolol chlorhydrate was rapidly desorbed from nanoparticles within the first 15 min.

In the case of suspension B, no significant desorption occurred within 90 min of dialysis. The

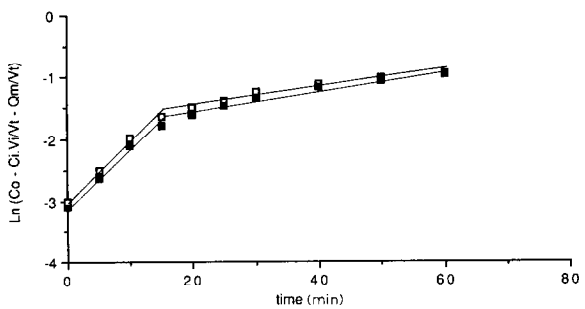

Fig. 6. Plots according to the transformation of kinetics (refer to text for details). ■, suspension A; □, suspension C.

energy of the ionic binding was supposed too high, therefore preventing the desorption process. It has been observed that the pharmacological response to the betaxolol chlorhydrate associated with nanoparticles in suspensions A, B and C was influenced by the physico-chemical characteristics of these preparations.

In comparison with Betoptic®, suspension B decreased the maximal intensity effect and induced a less prolonged response. This result could be related to the in vitro non-desorption of the drug which led to a minor amount of free drug available for transcorneal penetration (30% of the total drug concentration).

Despite the poor adsorption of the drug onto the nanoparticles, suspensions A and C appeared to induce an increased effect in terms of both time and intensity; however, because of a great interindividual variability in glaucomatous rabbits, the statistical analysis did not reveal a significant difference. Nevertheless, the reduction on the IOP of an experimentally induced glaucoma is the only way to measure the therapeutic effect of non-selective and β_1-selective blocking agents. Indeed, unlike β_2-selective blocking agents and cholinomimetic agents such as pilocarpine, the instillation of these drugs does not induce side effects such as mydriasis which can be correlated to the extent of their therapeutic activity.

An essential parameter for the preparation of a good nanoparticulate dosage form for ophthalmic purposes is that the drug should be released reasonably fast so as to display a therapeutic activity. Such a phenomenon was actually observed throughout the dialysis experiments in the case of suspension A as well as C, while it failed to occur in suspension B. It is obvious that the particles with the lowest drug payload but the less negative surface charge trigger off a better response than the particles containing a greater amount of drug adsorbed onto their surface but a more negative surface charge. Although they did not study the influence of the drug payload on the therapeutic effect, Harmia et al. (1986) showed that the effect of pilocarpine in eyes can be increased by adsorbing only 15% (the overall concentration of pilocarpine in the suspension is 2%, w/v) of this drug onto polybutylcyanoacrylate nanoparticles.

These results agree with the conclusions formulated by Fitzgerald et al. (1987) who showed that, using indium oxime linked to butyl-2-cyanoacrylate nanoparticles, the residence time of the carrier in the cul-de-sac was relatively short (corneal $t_{1/2} = 2.15$ min for nanoparticles vs. 1.3 min for indium oxime solution). This short residence time associated to a fast release of the drug might explain the increase of the ocular pilocarpine bioavailability (30%) in the experiments of Harmia et al. (1986) as well as the trend to prolong the betaxolol antiglaucomatous activity.

Therefore, the objective of getting the highest adsorption percentage of the drug onto the nanoparticles is far from being as indispensable a parameter since a relatively low amount of drug fixed onto the particles is enough the display a therapeutic effect which is found to be at least equivalent if not superior to that of the commercial eye drops. Moreover, it has been clearly demonstrated that the surface charge of the particles and the binding type between the drug and the particles, which determined the rate of desorption of the drug from the particles, was an outstanding parameter.

With a view to prolonging the entrance of drugs in the intraocular structures, a long residence time of the particles in the cul-de-sac and a total desorption of the drug from the particles during this time have to be associated. If the ability of the particles to improve the penetration of the drugs in the ocular tissues has been shown (Wood et al., 1985), further research needs to be accomplished in order to increase their own adhesive properties in the cul-de-sac.

Acknowledgements

The authors would like to thank Dr. G. De Burlet (Alcon-P.O.S. Laboratories, Kaysersberg, France) for his contribution to the in vivo experiments. The assistance of Miss A. Berthin in preparing the manuscript is gratefully acknowledged.

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