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The kinetics of in vitro release and the pharmacokinetics of miotic response in rabbits of gelatin and albumin microspheres with pilocarpine

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Summary

The present study had the purpose of evaluating a new ophthalmic drug delivery system, pilocarpine-loaded proteinaceous (gelatin or albumin) microspheres, aimed at ensuring a prolonged time of residence of the medication in the eye, as well as a better ocular bioavailability. The in vitro release data were analysed using different laws that can govern the release mechanism of drugs from microspheres. Application of the method of residuals to the experimental data indicated that the release was biphasic, regardless of the plotting method. The correlation coefficients are the best for a planar matrix mechanism in the case of gelatin and for a spherical matrix in the case of albumin microspheres. The aqueous suspension eyedrops of pilocarpine-loaded gelatin or albumin microspheres instilled in rabbit eyes prolong therapeutic effect compared to aqueous or viscous solutions of the free drug. The calculated pharmacokinetic parameters of the biological response show a lag time in the appearance of the miosis, a prolonged half-life of the input of response, a delayed time of the peak, but an enhanced intensity of the action when colloidal carriers are used.

Introduction

One of the major problems of ophthalmic therapy is to provide and maintain an adequate concentration of the drug at the site of action, for a prolonged period of time. The addition of suitable polymers to liquid ophthalmic vehicles is a common method for increasing the ocular contact time and hence the drug bioavailability (Saettone, 1982, 1984). However, the relatively low viscosity ophthalmic solutions generally do not have a sustaining effect. The ointments, gels or slowly dissolving lamellae remain in the eye for extended periods of time, but their usual high water content allows drug substances to diffuse quickly out of the system (Patton and Robinson, 1975; Bensinger et al., 1976; Katz and Blackman, 1977; Grass et al., 1984). A new approach to achieve controlled drug delivery is to use liposomes (Singh and Mezei, 1983; Stratford et al., 1983), nanoparticles (Wood et al., 1985) or microspheres (Beal et al., 1984) as drug carriers.

Due to the need for frequent dosing of pilocarpine from commercial solution preparations and because of extensive work that has been pub-

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lished regarding ocular absorption of pilocarpine from various systems of administration, this drug was selected as a model for the release study from microspheres obtained from natural polymers, gelatin or egg albumin, utilized as ophthalmic drug delivery systems. The present study had the purpose of evaluating the pilocarpine-loaded gelatin and albumin microspheres aimed at ensuring a prolonged medication in the eye. Our approach has been to prepare a suspension of polymeric microspheres of a sufficiently small size to be undetectable as particles by the eye, and big enough to resist drainage from the eye. This study is a part (LXXX) of our contributions to biopharmaceutical evaluation of drugs, the last paper in this field being on the epirubicin microspheres (Leucuţa et al., 1988).

Materials and Methods

Materials

Pilocarpine nitrate and other chemicals were either reagent or analytical grade. Sodium carboxymethylcellulose (Serva), gelatin (Merck) and egg albumin (Fabrica de Praf de Ouă, Arad, Romania) were used as received. Sunflower oil conformed to the Romanian Pharmacopoeia (IX ed.) requirements.

Preparation of solutions and microspheres

A 2% w/v solution of pilocarpine nitrate, made isotonic with sodium chloride, was prepared in pH = 7.4 Sorensen's phosphate buffer. A 2% w/v solution of pilocarpine in 1.4% carboxymethylcellulose in phosphate buffer was also prepared. For the preparation of gelatin microspheres 20 g of water were added to 8 g of gelatin. The swollen gelatin was warmed in a water bath at 55–60 °C with stirring, then 2 g of pilocarpine nitrate dissolved in 5 g water was added and the solution was poured into 75 g of sunflower oil containing 0.5% Span 80 previously heated to about 55–60 °C. The mixture was stirred for 5 min at 300 rpm, then the glass vessel was placed in an ice water bath and cooled to 5 °C. Dehydration was carried out by adding 50 g of isopropanol under stirring at 300 rpm for 30 min. The gelatin microspheres were separated by filtration, washed 3 times with isopropanol and air-dried. Sustained-release microspheres were obtained by maintaining microspheres for 24 h in a covered glass vessel saturated with formaldehyde vapours. The microspheres were allowed to air until the odor of formaldehyde was indetectable. For the preparation of the egg albumin microspheres 0.2 g of pilocarpine nitrate and 0.5 g of the egg albumin were each dissolved in 1 ml of distilled water and the solution was combined and mixed with 100 ml of 1% Span 80 in sunflower oil, then homogenized for 10 min. at 4500 rpm. The emulsion was added to an additional 100 ml of sunflower oil preheated to 150 °C, allowed to stand for 30 min with constant stirring, then cooled to room temperature. The microspheres were washed free of oil by adding ether, centrifuging for 10 min at 4500 rpm and decanting the supernatant. After the third wash the microspheres were allowed to dry in a desiccator.

Analysis of pilocarpine content in microspheres

Gelatin microspheres were allowed to stand in 5% hydrochloric acid at 55–60 °C for about 48 hours. Albumin microspheres were digested in 0.5 M acetic acid. The digested homogenates were centrifuged at 4500 rpm and the pilocarpine content in the supernatant was assayed by U.V. spectrophotometry at 215 nm.

Measurement of the release rate

About 0.01 g of gelatin microspheres or 0.007 g of albumin microspheres, each portion containing about 0.002 g pilocarpine nitrate, were suspended in 100 ml of distilled water at 22 ± 2 °C under stirring with a magnetic stirrer. Two ml of the dissolution medium samples were collected at scheduled intervals, filtered and analysed for their pilocarpine content by U.V. spectrophotometry at 215 nm.

Analysis of the in vitro data

For all sets of data where the curves of the percent released vs. time were biphasic, the method of residuals was applied (Leucuţa and Pop, 1981). The release data were analysed using different laws that can govern the kinetics of drug release

from microspheres: first order kinetics: $Q = f(t)$; $\ln(Q_0 - Q) = f(t)$; $dQ/dt = f(Q)$; matrix mechanism: $Q = f(\sqrt{t})$; $\ln Q = f(\ln t)$; $dQ/dt = f(1/Q)$; release from a spherical matrix: $\frac{3}{2}[1 - (1 - Q)^{2/3}] - Q = f(t)$; and zero order release: $Q_0 - Q = f(t)$, where Q_0 is original amount (%) of drug present in tested samples of microspheres, and Q is the amount (%) of drug released (Tomlinson et al., 1984; Schwartz et al., 1968; Higuchi 1961, 1963; Baker and Lonsdale, 1974; Benita and Donbrow, 1982; Samuelov et al., 1979; Hecquet et al., 1984; Gupta et al., 1986).

The coefficient of linear correlation (r) was calculated for each linear slope, for data analysis.

Biological studies

Male, albino New Zealand rabbits were used. The application of the dose (50 μl) was made on the everted lower lid of the right eye, using a syringe; the other eye served as control. The measurements of pupillary diameter were made with a micrometer, held at a fixed distance from the rabbits' eyes, by the same operator. All experiments were carried out in the same laboratory, with constant artificial lighting, after 30 min of acclimatisation of the animals in restraining boxes. After dosing the lids were held together for a few seconds in order to avoid loss of dosage form. Each pharmaceutical system was tested in 10 rabbits. The results are presented as average variation of pupillary diameter with respect to the basal diameter vs time.

Analysis of the in vivo results

The miotic effect of the pilocarpine drug delivery systems tested was pharmacokinetically evaluated. Exponential equations were least squares fitted to the means of pharmacological effect of pilocarpine in rabbit eyes, using a computer program for non-linear curve fitting. The magnitude and time delay of the peak effect of pilocarpine were obtained from actual data points. The magnitude of the effect was evaluated from the area under the curve (AUC) of the pharmacological effect using the trapezoidal rule (Leucuţa, 1981). The half-lives of the appearance (input) or disappearance (output) of miosis were calculated with the equation: $t_{1/2} = 0.693/k$ where k is the rate constant for the input (respectively output) of the biological response in the rabbit eyes. The relative magnitude of biological response was evaluated as follows: $B \cdot R_{rel} = AUC_{test\ product}/AUC_{aqueous\ solution} \cdot 100$.

Student's test was used to evaluate the statistical significance of the differences.

Results and Discussion

The gelatin microspheres as well as the albumin beads were recovered as a discrete free-flowing powder with spherical particle mean diameter below 30 μm (microscopically evaluated). The recovery of pilocarpine nitrate was almost complete: 20% in gelatin microspheres and 28% in albumin beads.

In vitro release studies

The profiles of pilocarpine release from hardened or non-hardened gelatin microspheres and from albumin microspheres are presented in Figs. 1 and 2. The release of pilocarpine from non-hardened gelatin microspheres follows first-order behaviour (Fig. 3) ($r = 0.9949$) with a rate constant of dissolution $k = -0.1193\ \text{min}^{-1}$ and a dissolution half-time $t_{1/2} = 5.8$ min. In vitro passive release of pilocarpine from monolithic hardened gelatin and albumin microspheres was biphasic with an initial large and fast release

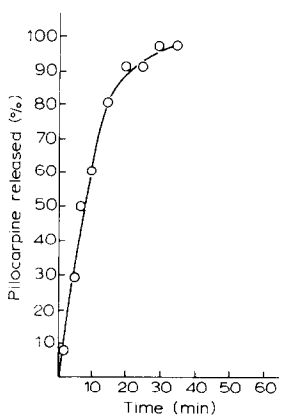

Fig. 1. The release profile of pilocarpine from gelatin microspheres.

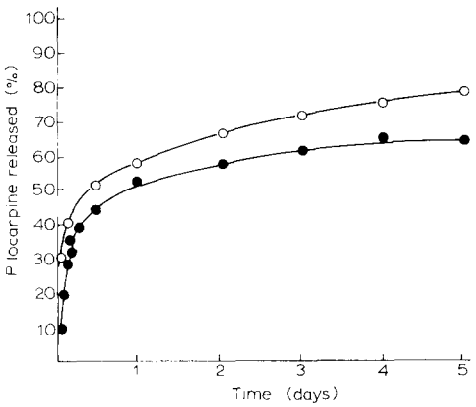

Fig. 2. The release profiles of pilocarpine from hardened gelatin (○) and albumin (●) microspheres.

followed by a much slower release. The duration of the fast release phase was almost 6 h, the slow release phase was prolonged over 5 days.

In Figs. 4–7 are presented the plots for pilocarpine released from gelatin and albumin microspheres according to different laws of release mechanism: first-order, planar matrix, spherical matrix, zero-order. Tables 1 and 2 show the equations for data analysis with several functions, as well as the linear correlation coefficients for the initial and terminal release phases of entrapped drug in hardened gelatin and in albumin microspheres, respectively. Application of the method of residuals to the experimental data indicated that the release was biphasic regardless of the plotting method. However, the differential forms of the rate equations, the logarithmic form of the

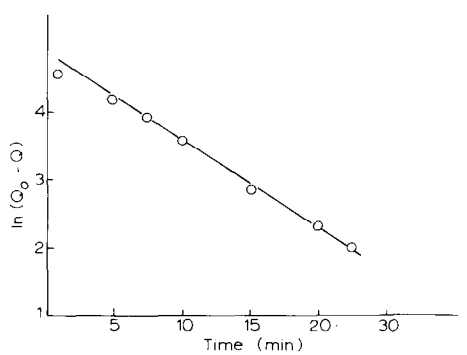

Fig. 3. Plot of $\ln(Q_0 - Q)$ vs time for pilocarpine released from gelatin microspheres.

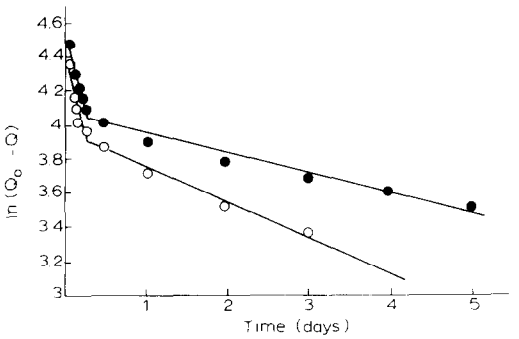

Fig. 4. Plot of $\ln(Q_0 - Q)$ versus time for pilocarpine released from hardened gelatin microspheres (○) and from albumin beads (●).

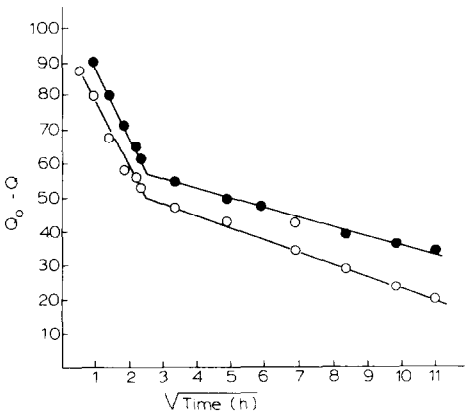

Fig. 5. Plot of $(Q_0 - Q)$ vs square root of time for pilocarpine released from hardened gelatin (○) and albumin (●) microspheres.

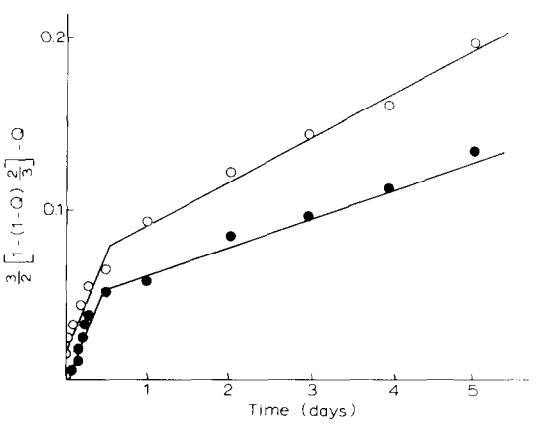

Fig. 6. Plot of $3/2[1-(1-Q)^{2/3}]-Q$ versus time for pilocarpine released from hardened gelatin (○) and albumin (●) microspheres.

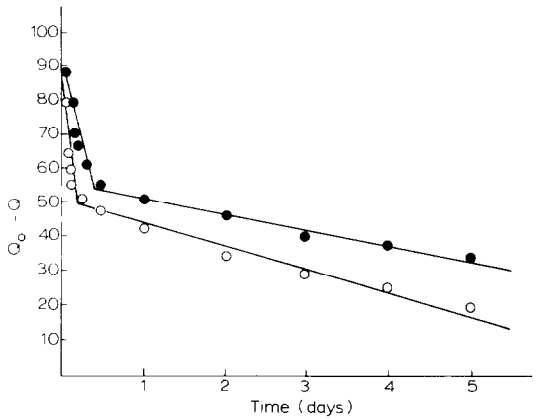

Fig. 7. Plot of $(Q_0 - Q)$ vs time for pilocarpine released from hardened gelatin (○) and albumin (●) microspheres.

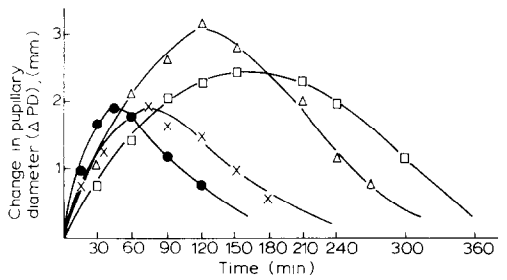

Fig. 8. Plots of miosis–time profiles. Mean values after equivalent doses of pilocarpine nitrate administered as aqueous eyedrops (●), aqueous viscous solution(x), aqueous suspension with hardened gelatin microspheres (△) and aqueous suspension with albumin microspheres (□).

diffusional equations, as well as the correlation coefficients, show that the kinetics of release obeys a planar matrix mechanism in the case of hardened gelatin microspheres and a spherical matrix mechanism in the case of albumin microspheres.

It has been reported that with albumin microspheres about 40% of the total drug is released rapidly and the remaining 60% is released slowly (Widder et al., 1979). Release of triamcinolone diacetate from human serum albumin microspheres has been reported to follow a first-order model (El-Samaligy and Rohdewald, 1982). More recently a biexponential first-order model has been employed to describe the drug release from albumin microspheres (Tomlinson et al., 1984; Gupta et al., 1986). The release of nitrofurantein from albumin beads complied to the dissolution model for a spherical matrix (Jun and Lai, 1983), the same being reported for the release of the metronidazole from magnetic microspheres and microcapsules of gelatine (Leucuţa, 1986).

Our experimental data demonstrate that the release mechanisms of pilocarpine from hardened gelatin microspheres or from albumin microspheres appear complex. The models identified can be employed in the study of the influence of different formulation factors on the drug release in order to obtain the desired characteristics. The biexponential function which provides an adequate fit to the experimental data set, $Q = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$, can be used to characterize drug release in the planar matrix as well as in the spherical matrix model. The two constants A and B are the zero time intercepts for initial and terminal phases and α and β are the apparent first order initial and terminal release rate constants, respectively.

TABLE 1

Mechanism of pilocarpine release from hardened gelatin microspheres

Model employed	Function	Equation	Correlation coefficients for initial and terminal release phases	
First order	$\ln(Q_0 - Q) = f(t)$	$Q = 0.56\ e^{-0.41t} + 3.94\ e^{-0.0022t}$	0.9193	0.9958
	$dQ/dt = f(Q)$		0.9959	0.9438
Planar matrix mechanism	$(Q_0 - Q) = f(\sqrt{t})$	$Q = 19.4\ e^{-0.96t} + 71.8\ e^{-0.11t}$	0.9458	0.9982
	$\ln(Q_0 - Q) = f(\ln t)$		0.9587	0.9967
	$dQ/dt = f(1/Q)$		0.9949	0.9923
Spherical matrix	$\frac{3}{2}[1 - (1 - Q)^{2/3}] - Q = f(t)$		0.8716	0.9696
Zero-order	$Q_0 - Q = f(t)$	$Q = 36.5\ e^{-0.42t} + 50.6\ e^{-0.0076t}$	0.8997	0.9804

TABLE 2

Mechanism of pilocarpine release from albumin microspheres

Model employed	Function	Equation	Correlation coefficients for initial and terminal release phases	
First order	$\ln(Q_0 - Q) = f(t)$	$Q = 0.56\ e^{-0.23t} + 4.0\ e^{-0.0011t}$	0.9475	0.9839
	$dQ/dt = f(q)$		0.9640	0.9491
Planar matrix mechanism	$Q_0 - Q = f\sqrt{t}$		0.9490	0.9987
	$dQ/dt = f(1/Q)$		0.7960	0.9704
Spherical matrix	$\frac{3}{2}[1 - (1 - Q)^{2/3}] - Q = f(t)$		0.9987	0.9976
Zero-order	$Q_0 - Q = f(t)$	$Q = 41.4\ e^{-0.26t} + 55.01\ e^{-0.0042t}$	0.9365	0.9675

TABLE 3

The equations which best fitted with the biological response (BR) of pilocarpine delivery systems studied

Formulation	Equation
Aqueous solution (1)	$BR = -3.437\ e^{-0.078t} + 3.52\ e^{-0.0136t}$
Viscous aqueous solution (2)	$BR = -4.004\ e^{-0.041t} + 4.029\ e^{-0.011t}$
Aqueous suspension of hardened gelatin microspheres (3)	$BR = -17.93\ e^{-0.020t} + 18.7\ e^{-0.0088t}$
Aqueous suspension of albumin microspheres (4)	$BR = -6.467\ e^{-0.0189t} + 6.54\ e^{-0.0064t}$

In vivo studies

The results of the miosis studies carried out in rabbits are presented in Fig. 8. The biological response was represented as the change in pupillary diameter (ΔPD) vs time. The pharmacological response in rabbits after the ocular administration of each of the 4 drug release systems experimented, was best fitted with the equations presented in Table 3. The calculated pharmacokinetic parameters of the biological responses are presented in Table 4.

The effect of pilocarpine is determined by its ocular absorption. The lag time in the appearance of the miosis is greater after colloidal systems than that of the aqueous solutions. The half-life of the input, $t_{1/2\ in}$, is 8.8 min after administration of the aqueous solution, whereas it was slower, 34 min and 36 min, after administration of the drug in the microsphere delivery systems. The time to peak of the biological response was significantly delayed ($P < 0.05$) but the intensity of the biological response was enhanced with the colloidal carriers, compared to aqueous solutions of the drug. The greatest biological response was observed after hardened gelatin microspheres, followed by albumin beads. The peak of miotic response of viscous solution was intermediate between those of the aqueous solution and of the polymer carriers. The areas under the curves of the biological responses vs time were roughly doubled and tripled after gelatin, respectively albumin, micro-

TABLE 4

The mean values of pharmacokinetic parameters of the biological response (BR) of pilocarpine delivery systems utilized in rabbit eyes

Formulation (see Table 3)	ΔPD_{max}	t_{max} (min)	t_{lag} (min)	$t_{1/2\ in}$ (min)	$t_{1/2\ out}$ (min)	AUC_0^∞ (ΔPD/h)	BR_{rel} (%)
(1)	2	45	11.4	8.88	50.95	196	100
(2)	1.88	75	12.6	17.11	67.28	281	143
(3)	3.1	120	18.4	34.47	78.75	446	227
(4)	2.5	150	32.4	36.66	108.28	642	327

spheres with respect to that of aqueous solution. The disappearance of the biological effect, $t_{1/2\,out}$, has values of 78 min and 108 min for the gelatin and albumin beads, respectively, and 50 min for the aqueous solution.

The pilocarpine release from microspheres has a first rapid phase with a rate probably greater than the rate of ocular absorption. The greater peak time of biological response compared with aqueous solution proves a prolonged contact with corneal surface. This may avoid ocular and systemic side-effects of the drug. The prolonged release of the second phase produced a much greater biological response for a prolonged period of time.

It was established that polyphthalamide microcapsules containing ^{99}Tc-labelled albumin remained longer than an aqueous solution of ^{99}Tc-labelled albumin in the rabbit eye (Beal et al., 1984). The aqueous albumin was rapidly cleared, only 10% of the instilled mass being present after 10 min. However, 90% of the instilled mass of microcapsules was present after 90 min (Beal et al., 1984).

It is evident from this preliminary study that microspheres can sustain the release of water soluble drugs to the precorneal area. Further studies are needed to modify the structure of the beads in order to have better control over the rate of release of entrapped pilocarpine in an efficient, economical and desirable manner.

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