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Systemic absorption of ocular pilocarpine is modified by polymer matrices

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Summary

Systemic absorption of ocularly applied pilocarpine (1.2 mg) was studied after administration in aqueous solution, in hydroxypropylcellulose (HPC) matrix, and in a matrix of *n*-butyl half-ester of poly(methyl vinyl ether/maleic anhydride) (PVM/MA). In vitro release of pilocarpine from the HPC-matrix deviated slightly and positively from the diffusional square root of time dependence. The rate of drug release was independent of the phosphate buffer concentration of the dissolution medium with an initial pH of 7.4; the rate of release was $10.91 \pm 0.59\%$ $min^{-0.5}$ in 1.3 mM buffer and $9.91 \pm 0.37\%$ $min^{-0.5}$ in 66.7 mM buffer. A matrix of *n*-butyl half-ester of PVM/MA released pilocarpine according to zero-order kinetics. The rate of drug release was $0.22 \pm 0.02\%$ min^{-1} in 1.3 mM phosphate buffer and $0.95 \pm 0.06\%$ min^{-1} in 66.7 mM phosphate buffer. From the 2% aqueous solution, pilocarpine was absorbed efficiently into the plasma ($t_{max} = 3.6 \pm 0.9$ min, $c_{max} = 0.384 \pm 0.024$ μg/ml). Pharmacokinetic analysis of data for drug absorption revealed that the conjunctiva of the eye was the most important site for systemic absorption of pilocarpine. Both the HPC matrix ($t_{max} = 35.0 \pm 7.9$ min, $c_{max} = 0.256 \pm 0.022$ μg/ml) and the matrix of *n*-butyl half-ester of PVM/MA ($t_{max} = 204 \pm 17.5$ min, $c_{max} = 0.112 \pm 0.014$ μg/ml) delayed and decreased the peak concentrations of pilocarpine in general circulation. ($AUC_{0-6\,h}/AUC_{0-6\,h,i.v.}$) values were 0.72 ± 0.08, 0.67 ± 0.16, and 0.41 ± 0.05 for the aqueous solution, HPC matrix, and *n*-butyl half-ester of PVM/MA matrix, respectively. During the in vivo study, HPC matrices dissolved in 7–12 min in the tear fluid. *n*-Butyl half-ester of PVM/MA neither dissolved totally nor released all the drug from the matrix in the tear fluid during 8 h. Besides improving ocular drug absorption, as shown in earlier studies, the pilocarpine concentrations in systemic circulation can be decreased by administering the drug in polymer matrices.

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Introduction

Ocularly applied aqueous solutions are drained from the conjunctival fornix via the canaliculi to the lacrimal sac and to the nasolacrimal duct where they have access to the nasal cavity and to the gastrointestinal tract (Trueblood et al., 1975). During the drainage process ophthalmic drugs may be absorbed into the systemic circulation and cause systemic side effects. For example, pilocarpine (Salminen et al., 1984), corticosteroids (Baba et al., 1983; Urtti and Salminen, 1984), β-blocking agents (Schmitt et al., 1981), and mydriatics (Anderson, 1980; Duzman et al., 1983) are absorbed in the systemic circulation after ocular administration.

Ophthalmic pilocarpine is absorbed into the eye mainly through the cornea (Doane et al., 1978). Less than 1% of the instilled dose is absorbed into the eye (Chrai and Robinson, 1974), however, owing to the barrier of corneal epithelium and to a rapid decrease in the amount and concentration of pilocarpine in the precorneal tear fluid (Sieg and Robinson, 1976; Lee and Robinson, 1979). Rapid precorneal loss of pilocarpine is mainly due to systemic absorption of the drug, drainage of the instilled solution, and lacrimation induced by the eye drops (Lee and Robinson, 1979; Thombre and Himmelstein, 1984). Thus, systemic absorption decreases the ocular bioavailability of pilocarpine and necessitates frequent administration of the drug, which increases side effects.

Precorneal retention and duration of the action of pilocarpine can be prolonged by administering the drug in water-soluble polymer matrices, which are generally placed into the lower conjunctival fornix (Maichuk, 1975; Salminen et al., 1983; Urtti et al., 1984a and b; Saettone et al., 1984). In a polymer matrix the drug is dissolved or dispersed in the polymeric material. When the matrix comes into contact with the tear fluid, the drug is released. Most reported polymer matrices of ophthalmic pilocarpine have been hydrophilic (Maichuk, 1975; Salminen et al., 1983; Saettone et al., 1984; Urtti et al., 1984a). Tear fluid, however, penetrates rapidly into hydrophilic polymers; and highly water-soluble pilocarpine diffuses rapidly from the matrix (Harwood and Schwartz, 1982; Urtti et al., 1984a). This results in prolonged pulse-entry of the drug into the eye. In such cases, ocular absorption of the drug is not controlled by the vehicle, and the increased ocular bioavailability is caused by prolonged precorneal retention of the drug. In addition to ocular bioavailability, peak drug concentrations and dose-related ocular side-effects are also increased (Salminen et al., 1983; Urtti et al., 1984b; Saettone et al., 1984).

Penetration of water into the matrix can be reduced by using hydrophobic polymers, e.g. alkyl half-esters of poly(methyl vinyl ether/maleic anhydride) (PVM/MA). In these polymers hydrophobic alkyl ester groups hinder the penetration of water into the matrix, while ionizable carboxylic groups make the surface of the polymer matrix water-soluble at pHs above the specific pH at which the polymer dissolves. The dissolution pH and hydrophobicity of the matrix are increased with increasing length of the ester group. Ideally, diffusion of the drug from the matrix is avoided, and the drug is released at the rate at which the polymer surface is dissolved (Woodruff et al., 1972; Heller et al., 1978).

Although ophthalmic vehicles with prolonged action have been studied extensively, their possible effects on systemic drug absorption have not been reported. A hydrophilic polymer (hydroxypropylcellulose; HPC), and *n*-butyl half-ester of PVM/MA have been suggested as potentially suitable support materials for ophthalmic drug inserts (Hussain et al., 1976; Katz et al., 1978; Saettone et al., 1984). The purpose of this study was to determine systemic absorption of pilocarpine in pigmented rabbits after the drug had been administered in aqueous solution and in matrices prepared from these polymers.

Materials and Methods

Materials

Adult pigmented rabbits (3.4–4.9 kg) of mixed breed were used in this study. Before the experiment the rabbits were housed singly in standard laboratory conditions: 10 h dark/14 h light cycle, 20.0 ± 0.5°C temperature, 55–75% relative air humidity. The test animals were given food and water ad libitum.

[^{3}H-G]Pilocarpine in ethanol solution was obtained commercially (Radiochemical Centre, Amersham, U.K.). The specific activity of the tracer was > 1 Ci/mmol, and the radiochemical purity was > 98%.

Poly(methyl vinyl ether/maleic anhydride) (PVM/MA) was obtained commercially (Aldrich Chemicals, Milwaukee, WI, U.S.A.). From the relative viscosity of 1% PVM/MA solution in methyl ethyl ketone, the molecular weight of the polymer was calculated to be about 360,000. The molecular weight of hydroxypropylcellulose (HPC) (Aldrich Chemicals) was about 100,000.

Synthesis of n-butyl half-ester of PVM/MA. PVM/MA (10.1 g) was incubated in 300 ml of *n*-butanol at the reflux temperature (117°C). The reaction was continued for 5 h until infra-red analysis showed a minimum amount of residual anhydride (Heilman, 1974). The butyl half-ester was precipitated with methanol–water (1 : 4) mixture. The precipitate was washed several times with boiling hexane, then dried and pulverized. The half-esterification was checked by dissolving the polymer samples in ethanol and titrating the solutions with 0.1 N sodium hydroxide to the phenolphthalein end-point.

Dosage forms

The pilocarpine base solution for intravenous administration (1.2 mg/ml) was made in sterile isotonic saline. Owing to the addition of pilocarpine, the osmotic pressure of the solution did not change appreciably. The ethanol solvent of [^{3}H]pilocarpine was evaporated by vacuum distillation, and the tracer was dissolved in the pilocarpine solution. This solution was sterilized by filtering it through a membrane with pore size of 0.2 μm (Gelman Sciences, Ann Arbor, MI, U.S.A.). The radioactivity of the final solution was 53 nCi/μl.

Isotonic aqueous 2% pilocarpine solution containing [^{3}H]pilocarpine was prepared according to the method of Salminen et al. (1984). This solution was buffered to pH 6.4 with phosphates, and its final radioactivity was 0.42 μCi/μl.

For both in vitro and in vivo studies, the polymer matrices were prepared using the solution casting method. For in vitro studies 2.575 g of HPC, 0.425 g of pilocarpine base and 30 μCi of [^{3}H]pilocarpine were dissolved in methanol and cast in a teflon-coated petri dish. Alternatively, 2.5 g of *n*-butyl half ester of PVM/MA, 0.5 g of pilocarpine hydrochloride and 30 μCi of tritiated pilocarpine were dissolved in acetone–methanol (1 : 1) mixture and cast as described above. The solvent was allowed to evaporate at room temperature. Circular matrices with a diameter of 13 mm and thickness of 0.331–0.425 μm were cut from the remaining films. For in vivo studies corresponding films with a smaller area and higher radioactivity were cast as described above. Matrices, each with a diameter of 4 mm, weight of 4.2 mg and radioactivity of 3.66 μCi (HPC) or 26.46 μCi (*n*-butyl half-ester of PVM/MA), were cut from the cast films.

Release studies

Pilocarpine release from the matrices was studied as described earlier (Salminen et al., 1983). Release of the drug from the polymer matrices was studied in 1.3 mM and in 66.7 mM phosphate buffers. Using sodium chloride, the buffer solutions were made iso-osmotic with tears. Initially they had a pH of 7.4 and temperature of 32°C. We investigated how the dissolution of *n*-butyl half-ester of PVM/MA and pilocarpine affected the pH of the buffer solutions.

In vivo study

During these experiments all test animals were kept in restraining boxes in a normal upright position. Each rabbit received 0.6 mg of pilocarpine base equivalents in both eyes. The aqueous solution (30 μl) was applied to the upper corneoscleral limbus and the polymer matrices in the lower conjunctival fornix. During the application the eye lids were gently pulled away from the globe and returned to normal position after application. Within 5 s of application 1.2 mg of pilocarpine was injected into the ear vein as a bolus.

Blood samples were taken from the cannulated ear artery of the rabbits at fixed times during the 8 h after application (6 h after i.v. injection). Plasma was separated and its radioactivity measured as described earlier (Salminen et al., 1984). In the text, drug concentrations are expressed as concentrations of total pilocarpine. The total pilocarpine concentrations include both the intact drug and possible metabolites.

Analysis of the results

To investigate the mechanism of drug release in vitro, the logarithm of the amount released (Q) was plotted against the logarithm of time (t) (Schwartz et al., 1968). When the slope (k) of the log–log plot is 0.5, the drug is released according to diffusion-controlled kinetics. If k is unity, the drug is released at a constant zero-order rate.

The magnitude and time delay of the peak concentrations of total pilocarpine were obtained from actual data points. The systemic availability of the radioactivity

borne by [^{3}H]pilocarpine was calculated as the area under the curve ($AUC_{0\text{-}6h}$) of the total pilocarpine concentration vs time, using the trapezoidal rule.

Exponential equations were least-squares fitted to the means of total pilocarpine concentrations in rabbit plasma after an intravenous injection, using a computer program for non-linear Gauss-Newton curve fitting. The residuals were weighted by using the inverse of plasma concentrations. As evidenced by the Akaike's Information Criterion (AIC), the best fit was obtained using a triexponential equation (Yamaoka et al., 1978). Pharmacokinetic microconstants were calculated for the open three-compartment model with elimination of the radioactivity from the central compartment. Microconstants and means of total pilocarpine concentrations were used for calculating the amounts of pilocarpine absorbed vs time plots. The amounts of total pilocarpine absorbed after administration in aqueous solution and in polymer matrices were calculated using a modification of the Loo-Riegelman (1968) method for a three-compartment open model (Boxenbaum and Kaplan, 1974).

Mann-Whitney's U-test was used to evaluate the statistical significance of the results.

Results

In vitro studies

The profile of pilocarpine release from the HPC-matrices deviated slightly from the diffusional square root of time dependence, i.e. $k = 0.609\text{–}0.658$ (Table 1). Neither the rate nor the mechanism of drug release was affected significantly by the phosphate concentration of the dissolution medium (Fig. 1, Table 1).

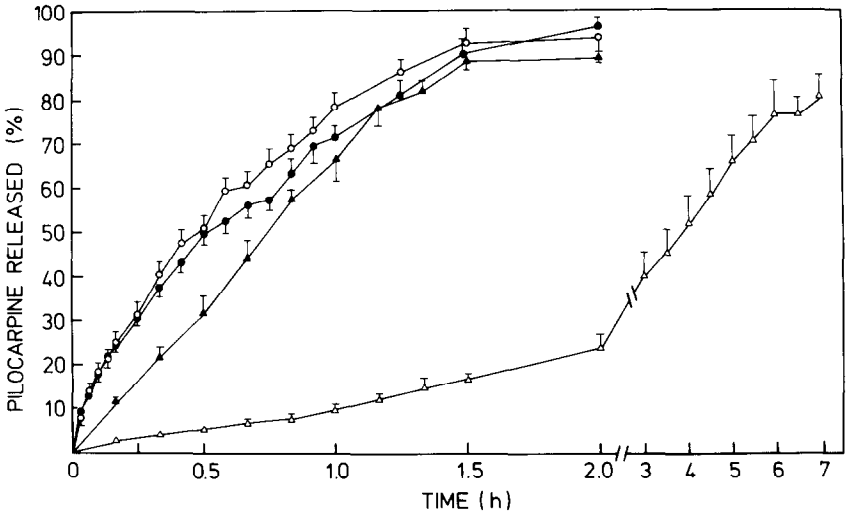

Fig. 1. Release of pilocarpine from the HPC-matrix (circles) and the *n*-butyl half-ester of PVM/MA matrix (triangles). Open symbols represent drug release in 1.3 mM and filled symbols in 66.7 mM phosphate buffers. Initially the pH of the buffers was 7.4. Means ±S.E. are presented.

Pilocarpine was released from the *n*-butyl half-ester of PVM/MA according to zero-order kinetics (Table 1, Fig. 1). The rate of drug release was increased ($P < 0.01$) by increasing the phosphate buffer concentration of the dissolution medium, whereas k-values were not significantly affected by the buffer concentration (Table 1).

Because of the different mechanisms of drug release, rates of release of pilocarpine from HPC and *n*-butyl half-ester of PVM/MA were compared by using t_{50}-values (Table 1). In 66.7 mM phosphate buffer the rate of pilocarpine release was slightly but significantly ($P < 0.05$) faster in HPC-matrices than in the matrices of *n*-butyl half-ester of PVM/MA. In 1.3 mM buffer solution the difference was larger ($P < 0.01$) (Fig. 1, Table 1).

In both 1.3 mM and in 66.7 mM buffer solution, HPC-matrices dissolved totally in 2 h. In 66.7 mM phosphate buffer, the matrices of *n*-butyl half-ester of PVM/MA dissolved during 2 h, but not during 7 h in 1.3 mM phosphate buffer. The pH of the 1.3 mM buffer solution decreased during the drug release (7 h) from the *n*-butyl half-ester of PVM/MA 0.9 pH units. The pH of the 66.7 mM phosphate buffer solution did not change during the release studies.

In vivo studies

Concentrations of total pilocarpine after i.v. injection were best fitted with the following equation: $C_p = 0.846e^{-43.210t} + 0.460e^{-1.609t} + 0.183e^{-0.047t}$, where C_p = concentration of total pilocarpine in plasma and t = time (h). The data points and the fit are shown in Fig. 2. The calculated pharmacokinetic parameters for the total concentration of pilocarpine in plasma were: $AUC_{0-\infty} = 4.196\ \mu g \cdot h/ml$, $V_p = 0.806$ l, $k_{12} = 22.373\ h^{-1}$, $k_{21} = 19.351\ h^{-1}$, $k_{13} = 2.310\ h^{-1}$, $k_{31} = 0.477\ h^{-1}$ and $k_{el} = 0.355\ h^{-1}$.

TABLE 1

PILOCARPINE RELEASE FROM POLYMER MATRICES IN PHOSPHATE BUFFER SOLUTIONS

Means ± S.E. of n determinations are presented.

Polymer	Phosphate concentration (mM)	Rate of release	$t_{50\%}$ [a] (min)	k [b]	n
HPC	1.3	10.91 ± 0.59 [c]	31.2 ± 3.6	0.658 ± 0.021	5
	66.7	9.91 ± 0.37	34.4 ± 2.3	0.609 ± 0.008	10
n-butyl half-ester of PVM/MA	1.3	0.22 ± 0.02 [d]	254.8 ± 23.8	1.047 ± 0.026	5
	66.7	0.95 ± 0.06 **	46.6 ± 3.5 **	0.946 ± 0.051	5

[a] $t_{50\%}$ = time of 50% drug release.
[b] k = slope of log Q vs log t plot (see Methods).
[c] $\%min^{-0.5}$ = percentage of the initial amount of drug.
[d] $\%min^{-1}$ = percentage of the initial amount of drug.
** $P < 0.01$ (Mann-Whitney's U-test), if compared to result for the same polymer in 1.3 mM buffer.

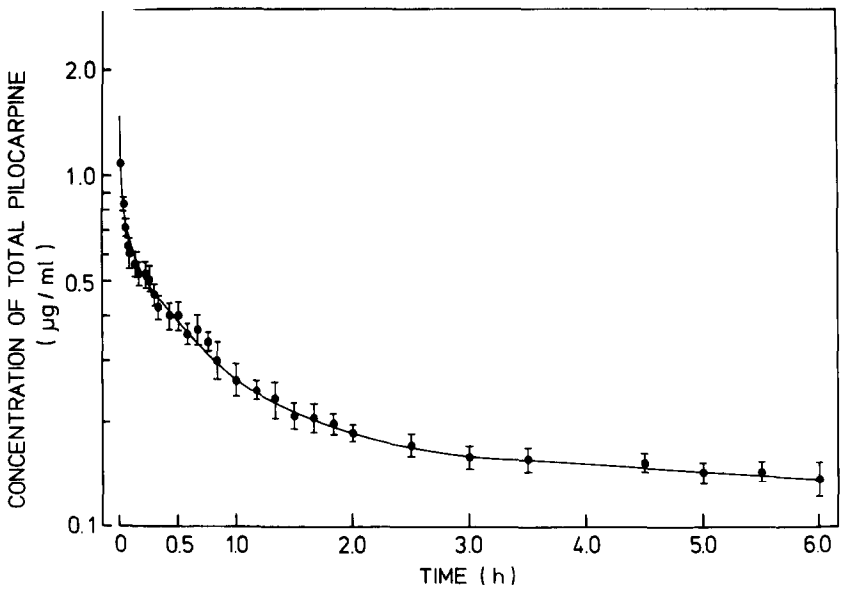

Fig. 2. Total pilocarpine concentrations in rabbit plasma after intravenous injection of 1200 µg of pilocarpine. Means ± S.E. of 5 determinations are presented.

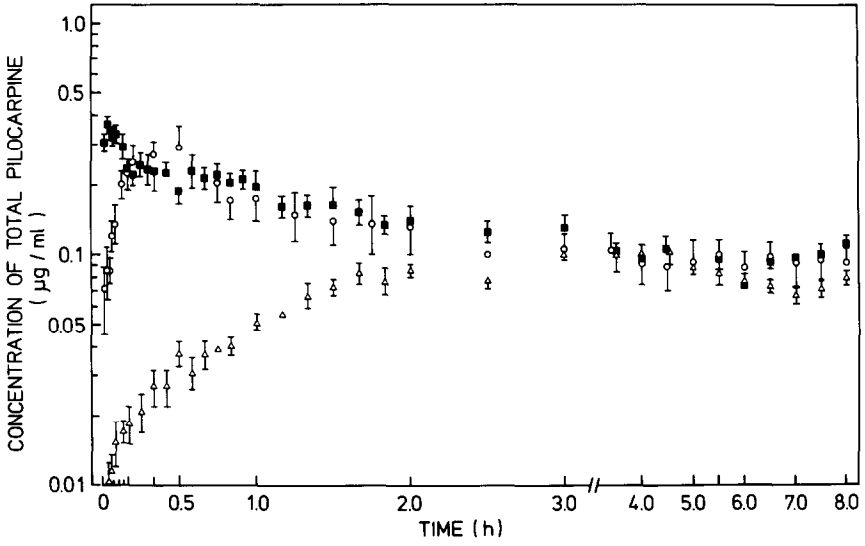

Fig. 3. Total pilocarpine concentrations in rabbit plasma after ocular administration of 1200 µg of pilocarpine in 2% aqueous solution (■), in HPC-matrix (○) and in a matrix of *n*-butyl half-ester of PVM/MA (△). Means ± S.E. are presented.

TABLE 2

PHARMACOKINETIC PARAMETERS OF TOTAL PILOCARPINE CONCENTRATION IN PLASMA AFTER ADMINISTRATION OF 600 μg OF PILOCARPINE TO BOTH EYES OF RABBITS

Means ± S.E. of n determinations are presented.

Dosage form	t_{max} [a]	c_{max} [b]	AUC_{0-6h} [c]	AUC_{rel} [d]	n
Aqueous solution	3.6± 0.9	0.384±0.024	0.833±0.087	71.6± 7.5	7
HPC-matrix	35.0± 7.9 **	0.256±0.022 **	0.777±0.184	66.8±15.8	5
Matrix of *n*-butyl half-ester of PVM/MA	204.0±17.5 **	0.112±0.014 **	0.473±0.054 **	40.7± 4.7 **	5

[a] t_{max} = time delay of peak concentration (min).
[b] c_{max} = peak concentration (μg/ml).
[c] AUC_{0-6h} = area under the concentration vs time curve (μg·h/ml).
[d] $AUC_{rel} = (AUC_{0-6h}/AUC_{0-6,i.v.}) \times 100\%$.
** $P < 0.01$ if compared to value of aqueous solution.

Pilocarpine was absorbed rapidly into the blood circulation ($t_{max} = 3.6 \pm 0.9$ min) after administration in an aqueous solution, whereas absorption was slower after administration of the drug in polymer matrices (Fig. 3, Table 2). The time of peak radioactivity in plasma was significantly delayed ($P < 0.01$) and its magnitude decreased ($P < 0.01$) with drug administration in the polymer matrices (Table 2). The time delay and magnitude of the peak radioactivity was changed more by *n*-butyl half-ester of PVM/MA than by HPC (Fig. 3, Table 2). The systemic availability of pilocarpine (AUC_{0-6h}) was not affected significantly by drug administration in HPC-matrices compared to aqueous solutions (Table 2). AUC_{0-6h} decreased ($P < 0.01$) when the drug was administered in *n*-butyl half-ester of

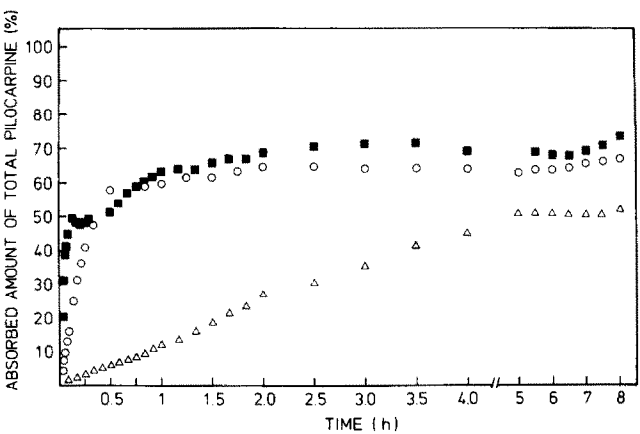

Fig. 4. Percent of pilocarpine absorbed as a function of time after ocular administration in aqueous solution (■), HPC-matrix (○) and in matrices of *n*-butyl half-ester of PVM/MA (△).

PVM/MA. Pilocarpine was not, however, released quantitatively from the *n*-butyl half-ester of PVM/MA matrix during 6 h.

After administration of pilocarpine in aqueous solution the drug was absorbed rapidly, but at a decreasing rate during the first 4 min (Fig. 4, Fig. 5). At 4 min the absorption rate decreased substantially, being about 0.008 min^{-1} for the next 55 min (Fig. 5). Then the rate of pilocarpine absorption again decreased until the absorbed amount reached a plateau at about 70% (Fig. 4, Fig. 5).

After ocular administration in HPC matrix, pilocarpine was absorbed at a rate of 0.038 min^{-1} during the first 12 min (Fig. 5). After 12 min the absorption rate decreased until it was about 0.023 min^{-1} between 12 and 30 min (Fig. 5). Thereafter the rate of drug absorption decreased again until the amount absorbed reached a plateau at about 65% (Figs. 4 and 5). After application, HPC matrices moistened instantly and dissolved in 7–12 min. Owing to the dissolution of the matrix, a viscous solution was formed in the conjunctival sac.

From the matrix of *n*-butyl half-ester of PVM/MA, pilocarpine entered the systemic circulation at a constant rate of 2.46 μg/min for 2 h. Thereafter the rate of pilocarpine absorption decreased continuously (Fig. 4). *n*-Butyl half-ester of PVM/MA matrices softened within minutes after application and maintained their shape and integrity for a few hours, after which the matrices jellified and swelled substantially. After 8 h the matrices were not totally dissolved.

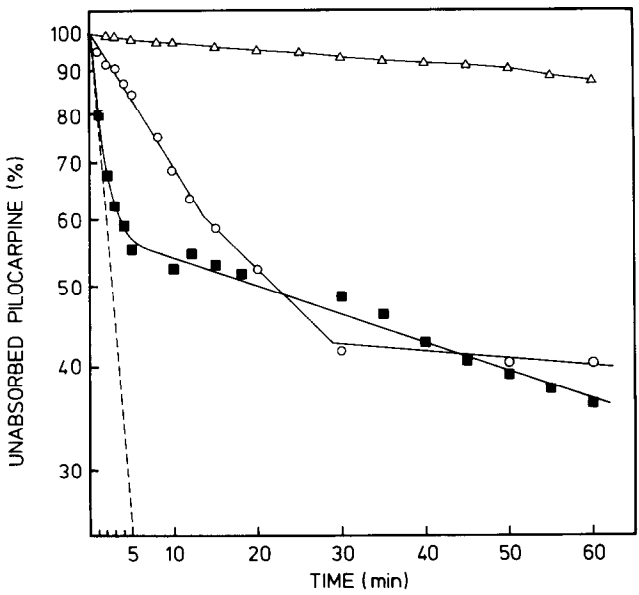

Fig. 5. Percentage of pilocarpine unabsorbed as a function of time after ocular administration of 1200 μg of pilocarpine in aqueous solution (■), HPC-matrix (○) and in matrices of *n*-butyl half-ester of PVM/MA (△).

In a separate test when the matrices were applied twice daily for one week, the matrices of HPC and of *n*-butyl half-ester of PVM/MA were not found to cause any irritation in the eyes of rabbits.

Discussion

Ophthalmic pilocarpine can be absorbed into the general circulation from the conjunctiva of the eye, lacrimal passages (canaliculi, lacrimal sac, nasolacrimal duct), nasal cavity, throat or gastrointestinal tract. Systemically, however, pilocarpine apparently is absorbed mainly from the conjuctiva of the eye; and other possible sites of drug absorption play a minor role in its systemic absorption.

Thombre and Himmelstein (1984) calculated a volume-independent rate constant, 8.84 μl/min, for elimination of pilocarpine from tear fluid to the systemic circulation through the conjunctiva of the eye. This rate constant was calculated on the basis of pilocarpine concentration in the tear fluid after administration of 0.2% pilocarpine solution. The value (8.84 μl/min) corresponds to a first-order rate constant of 0.236 min^{-1} when the volume of the donor solution is 37.5 μl (the instilled volume of 30 μl plus 7.5 μl, the volume of the tear fluid). Lee and Robinson (1979) stated that in the concentration range of 0.00002–2%, the first-order rate of precorneal loss of pilocarpine due to systemic conjunctival absorption is independent of the concentration of the instilled drug. Consequently, the amount of unabsorbed pilocarpine should decrease at a rate of 0.236 min^{-1} (the dotted line in Fig. 5), if the instilled solution of pilocarpine is retained in the conjunctival sac or if pilocarpine is absorbed at the same first-order rate in the drainage system as in the conjunctiva of the eye. Even though the absorptive area increases during drainage of the solution, the first-order rate of pilocarpine absorption decreased continuously (Fig. 5). The deviation from first-order kinetics during 4 min was evidently due to drainage of the instilled solution from the conjunctival sac. By that time drainage of the extra volume of solution has been virtually completed, and thereafter the volume of the precorneal fluid changes only slightly (Chrai et al., 1973). Consequently, drug absorption after 4 min roughly obeys first-order kinetics. The rate of drug absorption was about 0.008 min^{-1} between 4 and 60 min and after 60 min about 0.002 min^{-1} (Fig. 5). Although the exact rates of pilocarpine absorption from individual sites of absorption were not measured, compared to other possible sites, the conjunctiva of the eye is clearly superior for systemic absorption of ophthalmic pilocarpine.

About 1% of the instilled dose of pilocarpine is absorbed into the eye (Chrai and Robinson, 1974), and about 50% of the dose is absorbed into the systemic circulation from the conjunctiva (Fig. 4; Thombre and Himmelstein, 1984). The vast difference between amounts of drug absorption in the cornea and the conjunctiva is due to several factors. Firstly, the conjunctiva offers 16 times more area for drug absorption than does the cornea (Ehlers, 1965). Secondly, ophthalmic solutions stay in contact with the conjunctiva longer than with the cornea (Wilson et al., 1983). Thirdly, the corneal epithelium is a more effective barrier for drug absorption than

the conjunctival epithelium (Maurice and Mishima, 1984). The cells of the conjunctival epithelium are interconnected with junctions only in the apical cell layer, while in the basal portion of the epithelium the intercellular spaces are wide open (Steuhl and Rohen, 1983). Fourthly, because the conjunctival capillaries are highly fenestrated and are situated directly below the basal lamina of the conjunctival epithelium (Steuhl and Rohen, 1983), the flow of blood in the conjunctiva affects the systemic absorption of pilocarpine and other drugs. Precorneal loss of pilocarpine and glycerine from the tear fluid is substantially accelerated by vasodilators (histamine, pilocarpine) and retarded by vasoconstrictors (epinephrine) (Lee and Robinson, 1979). Thus, pilocarpine accelerates its own systemic absorption by dilating the conjunctival capillaries. Drug absorption is dependent on the blood flow, especially when the drug penetrates easily through the epithelial membrane (Winne, 1978). Accordingly, the results of Doane et al. (1978) indicate that the conjunctiva offers little resistance to the diffusion of pilocarpine.

In humans, Sorensen and Taagehoj Jensen (1979) have demonstrated that the conjunctiva of the eye is more permeable than other possible absorption sites. In spite of the decreased absorptive area, the rate of systemic absorption of pertechnetate was increased in patients who had had lacrimal sacs removed (0.021 min^{-1}) compared to normal individuals (0.014 min^{-1}). The highest rate of systemic absorption (0.027 min^{-1}), however, was found in patients with inflamed conjunctiva (intermittent red eyes) due to chronic dacryocystitis (Sorensen and Taagehoj Jensen, 1979). The possible importance of the nictitating membrane (which is negligible in humans) on systemic absorption of drugs is unclear, because no results dealing with the effect of the nictitating membrane on systemic absorption have been presented and the results concerning its effect on the precorneal drug loss and ocular bioavailability are conflicting (DeSantis and Schoenwald, 1978; Lee and Robinson, 1979; Mindel et al., 1984).

Owing to the high permeability of the conjunctiva of the eye, systemic absorption of drugs is probably increased when corneal and conjunctival contact of the ophthalmic solutions is prolonged. Ocular contact with solutions has been prolonged by applying eye drops in the lower conjunctival fornix (Fraunfelder, 1976), by closing the eye lids (Fraunfelder, 1976), and by increasing the viscosity of the instilled solution (Patton and Robinson, 1975; Wilson et al., 1983). For example, the increased systemic absorption of dexamethasone in suspension form, compared to solution form, is probably due to prolonged retention of the suspension particles in the conjunctival fornix (Urtti and Salminen, 1984).

In vitro release of pilocarpine was rather rapid and deviated slightly from the diffusional square root of time dependence (Table 1). Deviations of released amount vs square-root of time plots from linearity may be due to the initial swelling of the matrix (Korsmeyer and Peppas, 1981) or to dissolution of the polymer (Lapidus and Lordi, 1968).

Although pilocarpine was released rather rapidly from the HPC matrix in vitro (Fig. 1) and probably also in vivo (Fig. 4), systemic absorption of pilocarpine was controlled by the vehicle. This is owing to the rapidity of systemic conjunctival absorption in aqueous solution. In contrast, corneal absorption of pilocarpine after

administration in HPC-matrices is not controlled by the polymer (Urtti et al., 1984a; Saettone et al., 1984), because corneal absorption of pilocarpine from aqueous solutions is slow. The rate of systemic pilocarpine absorption after ocular administration was 0.038 min^{-1} during the first 12 min when the matrix was intact and was in the lower conjunctival fornix (Fig. 5). The decrease in rate of pilocarpine absorption after 12 min was due to dissolution of the matrix and to the subsequent flow of the viscous pilocarpine solution to the drainage system where systemic absorption of pilocarpine is slower. Thus, pilocarpine administration in an insoluble matrix with corresponding drug-releasing characteristics could lead to increased systemic absorption of the drug. Although the total pilocarpine concentrations after administration of eye drops and HPC matrix were about the same during most of the time interval studied (Fig. 3) and the absorbed amount of the drug was similar in these cases (Table 2), the peak concentrations of pilocarpine were reduced by 30% (Table 2). The steady-state pilocarpine concentration during chronic drug administration in HPC matrices is probably lower than during administration of eye drops, because in that case the retarded systemic absorption of the drug is combined with the less frequent dosing interval of the HPC matrices.

Monomer units of alkyl-esters of PVM/MA include a hydrophobic ester group and an ionizable carboxylic group with a pK_a-value of 6.0. The extent of ionization of carboxylic groups needed for polymer dissolution increases with increasing length of the ester side-chain. Thus, for each alkyl ester there is a specific dissolution pH above which that alkyl ester is soluble. The dissolution pH rises with increasing length of the alkyl ester; and for *n*-butyl half-ester, the dissolution pH is 5.1. Correspondingly, the rate of polymer dissolution at a certain pH decreases with increasing length of the ester group (Heller et al., 1978).

When a drug is released from a slab-shaped matrix at a rate controlled by the surface erosion of the matrix, the drug release obeys zero-order kinetics (Hopfenberg, 1976). Heller et al. (1978) showed that alkyl half-esters of PVM/MA release hydrocortisone at a constant zero-order rate, which is controlled by erosion of the polymer surface. In our study pilocarpine was also released according to zero-order kinetics from *n*-butyl half-ester of PVM/MA (Table 1). The release of pilocarpine was probably controlled by erosion of the polymer surface in 66.7 mM phosphate buffer.

The rate of pilocarpine release from *n*-butyl half-ester of PVM/MA was dependent on the concentration of the phosphate buffer in the dissolution medium (Table 1). The slow release of pilocarpine from the polymer in 1.3 mM phosphate was probably not due to the decrease in pH during the release study, because in that case the rate of drug release should decrease during the experiment. Based on the findings of Heller et al. (1978), it seems that the decrease in pH from 7.4 to 6.5 affects the erosion of *n*-butyl half-ester of PVM/MA only slightly. Increasing the buffer concentration of the dissolution medium increases the rate of drug release and polymer dissolution because the buffer carries the hydrogen ions (H^+) from the surface of the polymer matrix and diffusion of the dissociated polymer (P^-) is dependent on the flux of hydrogen ions by the requirement of charge neutrality (Heller et al., 1978). In 1.3 mM but not in 66.7 mM phosphate buffer the *n*-butyl

half-ester of PVM/MA swelled and a gel layer was formed on the surface of the matrix. Gelling of the polymer in the lower concentration of buffer and in decreasing pH might be due to the slow dissolution of the polymer surface compared to the penetration of the aqueous dissolution medium into the polymer. A more detailed study of the release kinetics of pilocarpine from alkyl half-esters of PVM/MA will be published later.

The approximate buffering capacities (P_i-values) of the tear fluid (Carney, 1979) and the 1.3 mM and 66.7 mM phosphate buffers were measured and calculated according to Skinner (1983). The buffering capacities of the tear fluid ($P_i = 0.0033$) and 1.3 mM phosphate buffer ($P_i = 0.0027$) were weak according to the scale used by Skinner (1983). The 66.7 mM phosphate buffer had a moderate ($P_i = 0.0200$) buffering capacity on the same scale. The rate of pilocarpine release from *n*-butyl half-ester of PVM/MA in vivo corresponds better to the in vitro release in 1.3 mM than in 66.7 mM phosphate buffer (Figs. 1 and 4). This applies to both the absolute rates of release and to the rates compared to HPC-matrices (Figs. 1 and 4). Other possible reasons for the slow release of pilocarpine in vivo include the decrease in pH of the tear fluid and lack of stirring. Formation of a gel layer around the matrix in vivo also indicates that the tear fluid has an insufficient buffering capacity.

The rate of pilocarpine release from *n*-butyl half-ester of PVM/MA is equal to or probably greater than the rate of pilocarpine absorption in the systemic circulation (148 μg/h). Thus, the rate of drug release from the matrices was enough to decrease the intraocular pressure, because Ocusert releases pilocarpine 40 μg/h and decreases the intraocular pressure. Consequently, polymer matrices with therapeutically adequate rates of pilocarpine release apparently modify the systemic absorption of the drug substantially (Fig. 3). This modification of the systemic absorption of pilocarpine by polymer matrices has clinical importance. Due to its rapidity and extent, the conjunctival systemic absorption of pilocarpine is also the most important factor in reducing the drug concentration — and the driving force of the transcorneal ocular absorption of the drug — in the precorneal tear fluid (Thombre and Himmelstein, 1984). Thus, systemic absorption of pilocarpine partly necessitates frequent application of concentrated pilocarpine solutions. This increases both ocular and systemic side-effects of the drug. According to Stafford (1981), pilocarpine causes side-effects in 81% of the patients; in 31% of the patients side-effects interfere with normal activity, and 14% of the patients give up the therapy due to the side-effects.

Perhaps systemic absorption of other ophthalmic drugs can be similarly modified with polymer matrices. Especially in children, the systemic side-effects of many ophthalmic drugs is a concern (for ref. see Palmer, 1982), because doses almost equal to those for adults are needed to obtain therapeutic concentrations of the drug in the eye (Patton and Robinson, 1976).

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