Controlled Delivery of Pilocarpine. 1. *In Vitro* Characterization of Gelfoam® Matrices

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Received October 21, 1991; accepted June 21, 1992

The potential of Gelfoam absorbable gelatin sponge as a carrier for ophthalmic delivery of pilocarpine was examined. Prolonged *in vitro* release of pilocarpine was achieved through pharmaceutical modification of the device by embedding a retardant in the pores. The device embedded with cetyl ester wax released pilocarpine in a zero-order pattern (release exponent = 0.93 ± 0.04) for up to 5 hr. This result corresponded well with a linear penetrant uptake by this device. The device impregnated with polyethylene glycol 400 monostearate exhibited anomalous drug transport with a release exponent of 0.63 ± 0.02. The absorption of water by this retardant and the formation of a gel layer on the surface slowed the penetration of the release medium into the deeper sections of the matrix, as well as the rapid outward diffusion of drug, resulting in a prolonged release of pilocarpine.

KEY WORDS: ophthalmic drug delivery; absorbable gelatin; zero-order release; prolonged release; pilocarpine.

INTRODUCTION

Pilocarpine is widely used as a topical miotic for the treatment of chronic, simple, or wide-angle glaucoma. However, following instillation of pilocarpine eyedrops, less than 1% of the instilled dose is absorbed into the eye (1). The dynamics of the lacrimal system results in the administered dose being eliminated from the precorneal area within 1 to 2 min of instillation (2). This necessitates frequent administration of large doses of pilocarpine to achieve the desired therapeutic effect. The need to instill pilocarpine four times daily has limited its usefulness and has contributed to patient noncompliance. Further, the frequent administration of large doses of pilocarpine results in a pulsed drug entry, with undesirably high concentrations in the aqueous humor immediately after instillation and subtherapeutic levels between pulses. This lowers the therapeutic efficacy of the drug and also results in side effects such as myopia and miosis (3). During the process of drainage from the precorneal area, pilocarpine is absorbed into the systemic circulation as well (4).

Ocular bioavailability and duration of action of pilocarpine can be increased by using drug carriers that retard precorneal drug loss and improve the corneal contact time (5). These vehicles include gels, latex systems, liposomes, nanoparticles, polymer matrices, and the Ocusert reservoir device (6). A drug carrier that also functions as a controlled-release system also reduces undesirably high pilocarpine concentrations in the eye and the systemic absorption of the drug. By delivering the drug at an optimal rate, these systems can achieve therapeutic efficacy with a minimum amount of drug and can reduce both ocular and systemic side effects.

Although various polymers can be used in the design of a controlled-release system, the use of a bioerodible or biodegradable carrier obviates the removal of the system from the eye at the end of its therapeutic lifetime and can minimize compliance problems. A drug delivery system which has all the above advantages could be a significant advance to ocular drug delivery.

Gelatin is a biopolymer which is widely available commercially and is very inexpensive. The water-soluble protein can be converted into absorbable gelatin by cross-linking the peptide chains via thermal treatment (7) without the use of cross-linking additives or by chemical treatment with cross-linking agents (8). The absorbable gelatin swells but does not dissolve in water. Water-swollen, cross-linked gelatin is a hydrogel, and therefore, it controls drug release by mechanisms typical of hydrogel-type delivery systems. In addition, due to its biodegradability, the absorbable gelatin device will not have to be removed from the conjunctival sac at the end of the drug dosing period.

The Gelfoam absorbable gelatin sponge is a medical device intended for application to bleeding surfaces as a hemostatic. It is a water-insoluble sponge prepared from purified pork skin Gelatin USP granules by a thermal treatment method (9). In this report, we describe a matrix device that (a) utilizes the potential of Gelfoam sponge as a biodegradable drug carrier for controlled ophthalmic delivery and (b) can be prepared using a simple fabrication method. The *in vitro* pilocarpine release and penetrant uptake profiles from these devices have been studied.

Pilocarpine, a common drug against glaucoma, was used in this study because its water solubility poses a challenge for effective delivery. In addition, the drug produces a response, i.e., miosis of the pupil, which can be readily monitored as an indicator of the drug levels in the aqueous humor.

MATERIALS AND METHODS

Materials

The drug carrier, Gelfoam sponge (compressed, size 100), was supplied by Upjohn Company (Kalamazoo, MI) and the drug, pilocarpine HCl, was obtained from Sigma Chemical Inc. (St. Louis, MO). The materials used as retardants, polyethylene glycol 400 monostearate (PEG-MS) and cetyl ester wax (CE-WAX), were obtained from Amend Drug and Chemical Company (Irvington, NJ). All other chemicals were of reagent grade and were obtained commercially.

Fabrication of Device

A matrix of 4 × 4 × 2 mm was cut from a slab of

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0724-8741/93/0100-0109$07.00/0

Gelfoam sponge and was allowed to soak in a solution of 25.5 mg pilocarpine (equivalent to 30 mg pilocarpine HCl) in chloroform. The solvent was evaporated slowly under nitrogen in an analytical evaporator (The Meyer N-Evap, Oragnomation, Inc.).

To prepare matrices containing a retardant, the retardant was dissolved in chloroform along with the drug and the matrices were prepared as per the procedure described above. After complete evaporation of the solvent, the dried Gelfoam–drug–retardant system was weighed using a Mettler (Model AE163) analytical balance. Preliminary experiments were carried out by embedding varying amounts (2 to 20 mg) of retardant to determine the optimum level needed in the devices. The results of the experiments, including a microscopic observation and a measurement of pilocarpine release profiles, suggested that an optimum of 8 mg of retardant would be required to occlude the interstices in the Gelfoam matrix remaining after embedding the drug.

Drug Release

The *in vitro* release profile from the dried matrix was measured using the rotating-bottle method (NF XIV). The matrix was transferred to a 5-ml screw-capped glass vial containing 3 ml of release medium, double-distilled water at 25°C. The vial was rotated end over end at 16 rpm (Labquake Shaker, Labindustries, Inc.). At various intervals up to 48 hr, the release medium was withdrawn completely and immediately replaced with an equal volume of fresh release medium. The samples were adjusted to a pH of 2.5 with 1 *N* HCl and analyzed for pilocarpine hydrochloride by HPLC. Three or more independent measurements of drug release profiles were made and an average value was calculated for time point. From the total amount of pilocarpine released, and knowing the total weight of the device and the weight of the Gelfoam backbone, the amount of retardant actually embedded in the device was calculated.

HPLC Assay of Pilocarpine Hydrochloride

The samples were analyzed for pilocarpine content using a modification of the method reported by Drake *et al.* (10). Separation was achieved by isocratic reversed-phase chromatography (Beckman Model 332 LC system with a Model 165 detector) using a Lichrosorb RP-18, 10-μm column (Alltech/Applied Science) at a flow rate of 1 ml/min at ambient temperature. The mobile phase was prepared by mixing 850 ml of 5% monobasic potassium phosphate solution in water (pH adjusted to 2.5 with 85% phosphoric acid) and 150 ml of methanol. The eluant peak was detected by measuring absorbance at 216 nm. The retention time was 5.5–6 min.

Analysis of *in Vitro* Release Data

The fraction of pilocarpine released from the matrices, M_t/M_∞, was analyzed using the relationship (11)

$$\frac{M_t}{M_\infty} = kt^n \qquad (1)$$

where k is a kinetic constant and n is a release exponent, both being characteristic of the matrix–eluant system, and t is the duration of release. The release exponent n characterizes the mechanism of release: $n = 0.5$ indicates release by a diffusive (Fickian) mechanism, $n = 1$ is characteristic of zero-order drug release, and $0.5 < n < 1$ indicates anomalous diffusion.

Penetrant Uptake

The dynamic swelling behavior of the pilocarpine delivery systems was studied by allowing initially dry devices to swell in distilled water. The matrices, prepared by the procedure described above, were enclosed in 5-ml vials containing 3 ml of distilled water. At various intervals, the matrices were withdrawn, the adhering water droplets were blotted with an absorbent wiper (Kimwipes, Kimberly-Clark, Roswell, GA), and the swollen matrices were weighed using a Mettler (Model AE 163) analytical balance ($\pm 1 \times 10^{-4}$ g). The matrices withdrawn as samples were then discarded and fresh samples were used for further time points. The extent of water uptake was expressed as the weight of water absorbed by a unit weight of the original, dry matrix multiplied by 100 (% water uptake). The results are plotted as the average of triplicate measurements.

RESULTS AND DISCUSSION

Drug Release

Figure 1 shows the plot of percentage pilocarpine released as a function of time from a simple Gelfoam matrix and from the matrices embedded with CE-WAX and PEG-MS as retardants. The simple Gelfoam device released most of the embedded drug released, within 15 min. The Gelfoam sponge matrix has a network of interstitial pores through which the release medium penetrates the matrix interior and leaches out the water-soluble drug, pilocarpine. The almost-instantaneous release of the pilocarpine (>90% in 15 min) from the Gelfoam matrix indicates that sink conditions were appropriately maintained in this system. Furthermore, all the embedded pilocarpine was released from the matrix, which suggests that the drug was not retained via irreversible binding by the Gelfoam backbone.

In order to prolong the release of the drug, the intersti-

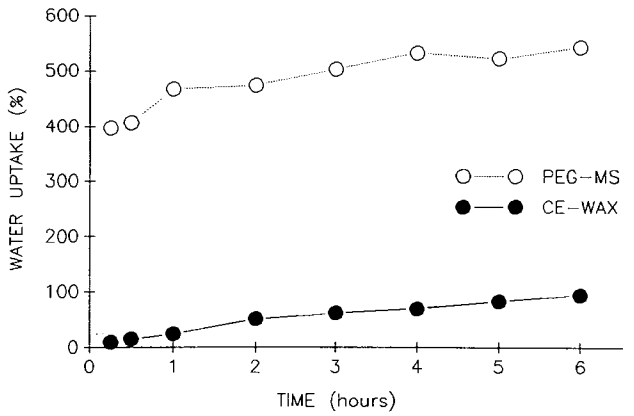

Fig. 1. Release of pilocarpine from Gelfoam, PEG-MS, and CE-WAX matrices.

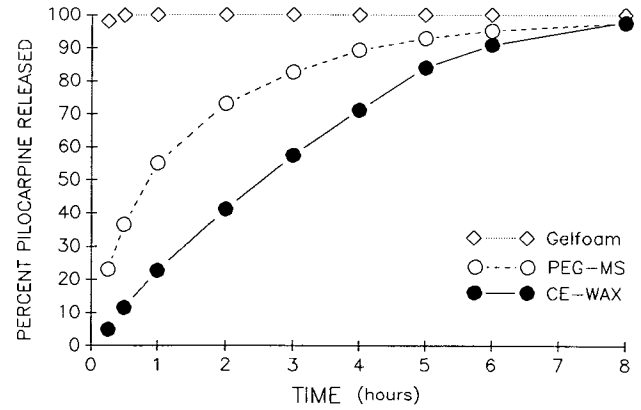

Fig. 2. Percentage released versus square root of time plot for PEG-MS matrix.

tial pores left in the matrix after impregnating pilocarpine were occluded by embedding various retardants to inhibit the leaching action of the water. The materials used as retardants included polydimethylsiloxane fluids, polyethylene glycol 400 monostearate (PEG-MS), polyethylene glycol 400 distearate, glyceryl monostearate, stearyl alcohol, white wax, and cetyl ester wax (CE-WAX). However, for the purposes of conciseness and clarity of presentation, the results of *in vitro* evaluation of the two most effective retardants, CE-WAX and PEG-MS, are presented here.

CE-WAX, used as a stiffening agent in topical preparations, has a melting range of 43–47°C and is practically insoluble in water (≤0.01 mg/ml). It exhibits an extremely low order of toxicity, and in the Draize eye irritation test in rabbits it produces no to mild irritation of extremely short duration (12). PEG-MS, a nonionic o/w emulsifier, is a waxy cream with an HLB value of 11.1 and is dispersible but not soluble in water. In acute eye test on rabbits PEG-MS was found to be a nonirritant (12).

A comparison of the drug release profiles from the two modified Gelfoam matrices embedded with retardants with that from the simple Gelfoam matrix (Fig. 1) indicates that embedding the retardants in the matrix is an effective means of prolonging drug release. The release of pilocarpine from the CE-WAX device very nearly approximates a linear, zero-order profile for up to 5 hr. When a porous polymeric

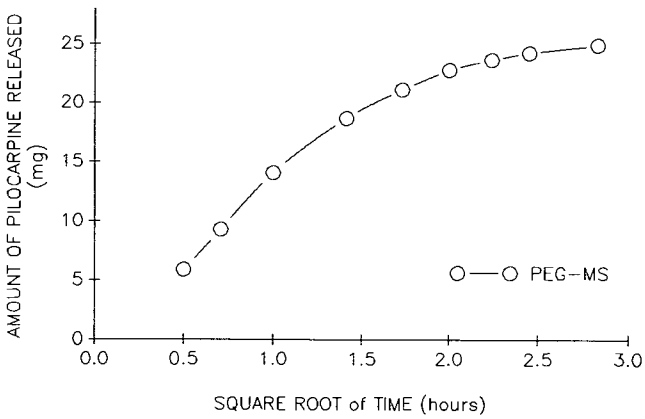

Fig. 3. Plot of ln (fractional drug release) versus ln (time) for PEG-MS and CE-WAX matrices.

Table I. Pilocarpine Release Parameters for PEG-MS and CE-WAX Matrices

Gelfoam device with retardant	Kinetic constant k	Release exponent n	r^2
PEG-MS	0.556 ± 0.012	0.628 ± 0.021	0.999
CE-WAX	0.204 ± 0.020	0.934 ± 0.036	0.996

matrix carrying a highly water-soluble solute is introduced in the release medium, the medium penetrates the drug-filled pores by dissolving the drug, which is then released in the external medium by molecular diffusion (13,14). The rate of penetration of the medium into the matrix depends on the tortuosity of pores in the matrix and the solute density, solubility, and diffusion coefficient in the solvent. De Haan and Lerk (15) studied drug release from a device comprising two structures: the housing phase, which dissolves in the penetrating solvent, forming solvent-filled channels, and simultaneously exposes an increasing surface area of the drug-containing restraining phase to the solvent. The device delivers most of its drug content at a zero-order rate. The zero-order release of pilocarpine CE-WAX device could be the result of a constant rate of progressive hydration of the interspersed drug particles by the penetrating medium.

The drug release from the PEG-MS device is not linear are expected for the diffusional square root of time dependence (Fig. 2). The deviations of the amount released vs square root of time plots from linearity may be due to the swelling of the matrix (16) or the dissolution of the polymer (17). However, no dissolution of the matrix was observed during the experiment.

To analyze the pattern of drug release from the two devices, the data were fitted to Eq. (1) and are presented as a ln–ln plot of fractional pilocarpine release versus time in Fig. 3. The values of the kinetic constant (k) and the diffusional exponent (n) are listed in Table I. The analysis of the profile from the PEG-MS device suggests an anomalous nature of drug transport (n = 0.628 ± 0.021), whereas the CE-WAX device released pilocarpine according to near zero-order kinetics (n = 0.934 ± 0.036).

Because of their differing *in vitro* drug release profiles, the efficacies of the retardants used in controlling the release of pilocarpine were compared by using t_{50} and t_{90} values, the times required for 50 and 90% drug release, respectively (Table II). The t_{90} values for the devices embedded with PEG-MS and CE-WAX were 4.58 and 5.72 hr, respectively. In a subsequent study, the values of these parameters will be used as a basis for comparing the *in vitro* release patterns with the duration of the pharmacological effect of pilocarpine achieved with these devices in rabbits.

Table II. Duration for 50% (t_{50}) and 90% (t_{90}) Release of Pilocarpine

Retardant	t_{50} (hr)	t_{90} (hr)
PEG-MS	0.85 ± 0.03	4.58 ± 0.67
CE-WAX	2.61 ± 0.34	5.72 ± 1.05

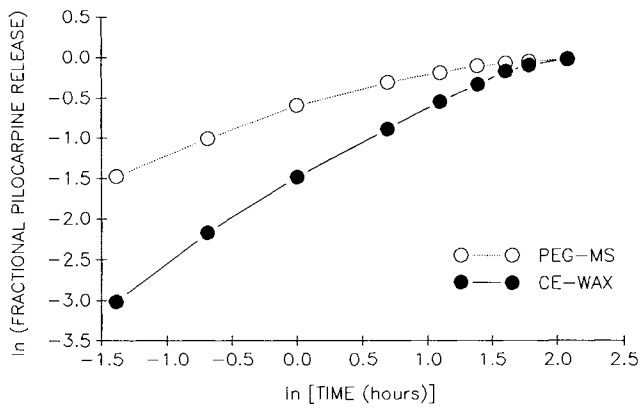

Fig. 4. Dynamic penetrant (water) uptake by PEG-MS and CE-WAX matrices.

Penetrant Uptake

Since prolonged drug release from the Gelfoam device is achieved by embedding retardants in the interstitial pores of the matrix to inhibit the penetration of water into the pores and simultaneous outward diffusion of pilocarpine, the water uptake by the two devices was measured.

As indicated in Fig. 4, the PEG-MS device absorbs water at a very high initial rate and quickly reaches a near-equilibrium value. Despite this, the device yielded a prolonged release of pilocarpine. PEG-MS is a hydrophilic polymer which exhibits a capacity to absorb water and form a gel. This gel formation can effectively reduce the penetration of water into the matrix interior and the diffusional release of the drug. Thus, the swelling of PEG-MS in the release medium to form a gelatinous hydrated layer on the surface of the matrix could explain the prolonged release of pilocarpine via an anomalous transport mechanism from the device.

Figure 4 also shows the pattern of water uptake by the CE-WAX matrix. The percentage water uptake increases nearly linearly with time. This corresponds well with the near-zero-order drug release from this device.

The overall results of the study indicate that the absorbable gelatin sponge, Gelfoam, is an excellent drug carrier for prolonged opthalmic delivery of pilocarpine. The matrix-type device is easy to fabricate and inexpensive to manufacture. Prolonged release of a highly water-soluble drug, pilocarpine, from the porous, hydrophilic biopolymer may be achieved by occluding the pores in the matrix. Using this approach, drug release from the device can be modulated without any chemical alteration of the protein. Embedding a hydrophilic polymer (PEG-MS) with gel-forming capabilities in the pores results in the anomalous drug release. On the other hand, the use of a hydrophobic material (CE-WAX) as a retardant yields a near-zero-order drug release profile of pilocarpine.

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