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Controlled delivery of pilocarpine. 2. In-vivo evaluation of Gelfoam® device

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Abstract

In this report, an ocular device for the controlled delivery of pilocarpine was evaluated in albino rabbits using miosis as a bioassay for efficacy. The device was fabricated using Gelfoam® (absorbable gelatin sponge, USP) in the form of a matrix system. The efficacy of the device was compared in a cross-over study to the two conventional ophthalmic pilocarpine dosage forms, the eyedrop and the gel. The in-vivo results show that the gelfoam device is more effective than the two conventional pilocarpine dosage forms in prolonging the duration of the pilocarpine activity. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Most ophthalmic drugs are administered topically in the form of eyedrops. Although convenient and inexpensive, this type of delivery system yields low therapeutic efficacy due to the dynamics of the lachrimal system (i.e. blinking, lachrimal secretion and nasolachrimal drainage). The low efficacy necessitates more frequent administration to achieve the desired therapeutic effect. This can increase the frequency and severity of both ocular and systemic side effects. Therefore, it is necessary to develop safer, efficacious and more acceptable ocular delivery systems. Delivery systems that are capable of releasing the drug in a prolonged manner are of interest because they can improve the ocular residence time. An increase in ocular residence time maximizes the duration for topical or local action and also minimizes the systemic side

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effects. Additionally, a controlled release preparation requires fewer instillations and therefore will lead to increased patient compliance.

Several preparations have been investigated for prolonging the delivery of topically applied drugs to the eye. These preparations include ointments (Sieg and Robinson, 1979; Saettone et al., 1980), liposomes (Benita et al., 1984; Taniguchi et al., 1988; Meisner et al., 1989), nanoparticles (Diepold et al., 1989), emulsions (Naveh et al., 1994), gels (March et al., 1982; Deshpande and Shirolkal, 1989; Cohen et al., 1997) and ocular inserts (Armaly and Rao, 1973; Maichuk, 1975; Grass et al., 1984; Urtti et al., 1984; Saettone et al., 1990; Sasaki et al., 1993). For excellent review on these subjects see Shell (1984), Lee and Robinson (1986), Le Bourlais et al. (1995) and Gurtler and Gurny (1995). Ocular inserts can be further categorized into non-biodegradable (non-erodible) and biodegradable (erodible). The non-erodible inserts such as Ocusert® possess several drawbacks. These include irritation, difficulty in proper retention of the device in the cul-de-sac, and the need for removal from the eye at the end of dosing. The use of biodegradable inserts is preferable because they obviate the need for the removal of the device from the eye at the end of dosing. Furthermore, they can be easily removed at anytime if desired. If these devices soften upon contact with tear fluid, they will be more comfortable and better retained in the cul-de-sac.

In the previous report, Nadkarni and Yalkowsky (1993) described the fabrication of a bioerodible eye insert in the form of a matrix system for the controlled delivery of pilocarpine. Commercially available Gelfoam® (absorbable gelatin sponge, USP) was utilized as the drug carrier. The in-vitro prolonged release of pilocarpine from the device was accomplished by embedding a retardant in the matrix. It was found that the embedment of the Gelfoam® pores with certain type of retardant is an effective way of controlling the release of the drug without altering the biodegradability of the gelatin. Various retardants were screened and evaluated in-vitro for their ability to prolong the release of pilocarpine from the sponge based on the release rate profiles. A device embedded with Cetyl ester wax (CEW) was found to be the most effective. It was shown to give a near-zero-order release pattern of pilocarpine in-vitro (Nadkarni and Yalkowsky, 1993).

In this report, the proposed device embedded with CEW is evaluated in-vivo using the albino rabbit as a model. The controlled delivery device is compared with two commercial ophthalmic pilocarpine dosage forms, an aqueous solution (Pilocar®) and an aqueous gel (Pilopine HS®), in a cross-over study. The pharmacological response produced by pilocarpine (i.e. miosis of the pupil) is used to assess the relative efficacy of the ocular pilocarpine delivery systems. The advantage of using the pharmacological response are the simplicity of measuring the pupil diameter and the fact that it is non-invasive.

2. Experimental section

2.1. Materials

Gelfoam® (absorbable gelatin sponge, USP, size 100) was obtained as a gift from the Pharmacia-Upjohn Company (Kalamazoo, MI). (+)-Pilocarpine HCl was purchased from Aldrich Chemical Company, Milwaukee, WI). Cetyl ester wax (CEW) was obtained from Amend Drug and Chemical Company (Irvington, NJ) and used as a retardant. All other chemicals were of reagent or HPLC grade and were obtained commercially and used without further purification.

2.2. Fabrication of the controlled delivery device

The controlled delivery device was prepared according to the procedure described earlier (Nadkarni and Yalkowsky, 1993). Briefly, a Gelfoam® matrix of $2.5 \times 2.5 \times 1.0$ mm was cut from a slab of the Gelfoam® sponge and accurately weighed with a Mettler (model AE163) analytical balance. A total of 6.0 mg of pilocarpine hydrochloride (equivalent to 5.1 mg of pilocarpine) along with 3.0 mg of Cetyl ester wax were dissolved in chloroform. The mixture was placed on the Gelfoam® matrix by means of Pipetteman pipettor and sorbed into the matrix. The solvent was evaporated slowly under nitrogen

in an analytical evaporator (the Meyer *N*-Evap, Oragnomation). After complete evaporation of the chloroform, the dried device containing pilocarpine and retardant was weighed to verify its content.

2.3. In-vivo evaluation

The therapeutic efficacy of the Gelfoam® device embedded with Cetyl esters wax (CEW) was compared with that of two commercial ophthalmic pilocarpine preparations, Pilocar® (6% pilocarpine HCl ophthalmic solution, CooperVision Pharmaceuticals) and Pilopine HS® gel (4% pilocarpine HCl Sterile Ophthalmic Gel, Alcon Laboratories) in a cross-over experiment.

Six New Zealand white rabbits of either sex weighing 6–7 lb. (Myrtles's Rabitry, Thompson Station) were used in the cross-over study. A washout period of at least 5 days was allowed between experiments. Rabbits were kept in restraining boxes during the experiments. All animals were conscious and their heads were unencumbered so that all normal head and eye movements were maintained. One hour prior to the experiments the animals were placed in the laboratory for acclimatization to the light, temperature and humidity.

The pharmacological activity of pilocarpine (i.e. pupil constriction) is monitored to assess the therapeutic efficacy of different ocular delivery systems. The pupil diameters of both eyes of each rabbit were measured prior to the instillation of the delivery systems and throughout the experiment. Equivalent amounts (5.1 mg) of pilocarpine in three delivery systems were instilled in one eye while the other eye was used as a control. For instillation of the delivery systems, the lower eyelid was pulled slightly away from the globe and either the eyedrops or the eye device was instilled in the center of the lower conjunctival cul-de-sac with care to avoid direct contact with the eye. The lower eyelid was returned to its normal position immediately following instillation of either eyedrops or the eye device.

The pupil diameters of both eyes were subsequently measured at predetermined time intervals by using the pupil gauge shown in Fig. 1. For the measurement, the gauge was brought forward slowly from the back of the eye and was held at a fixed distance from the eyeball for at least 30 s before taking the measurement of the pupil diameter. The miosis response (constriction in pupil diameter) was calculated from the difference between the pupil diameter of the control eye from that of the experimental eye at a given time. The baseline response values for the CEW device was obtained by instilling either Gelfoam® sponge containing the retardant alone (Placebo) or simple Gelfoam® sponge.

3. Results

The results of the three ocular delivery systems containing equivalent amounts of pilocarpine (5.1 mg) are shown in Fig. 2. The figure shows the plots of the mean miosis response (changes in pupillary diameter) as a function of time following the instillation of the three pilocarpine ocular delivery systems. As stated above, the experiment was done in a cross-over fashion and the miosis response is assumed to represent the pilocarpine ocular efficacy. It can be seen from the figure that the instillation of the eyedrop solution results in a rapid increase in the miosis response followed by a rapid return to normal. The increased miosis response returns to normal in 4 h. The gel formulation also produces a rapid increase in miotic effect. However, as can be seen from the figure the return to the baseline level is slower (6 h). On the other hand, the instillation of the CEW device results in a somewhat more gradual increase of miosis response and a more substantial prolongation of the miotic response than the same dose administered as solution or gel. The miosis response returns to the baseline level in approximately 12 h. The instillation of either the placebo device or simple Gelfoam® sponge did not produce noticeable change in the pupil diameter.

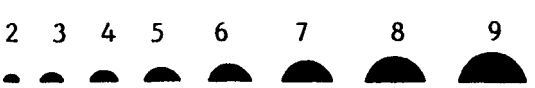

Fig. 1. Pupil gauge (mm).

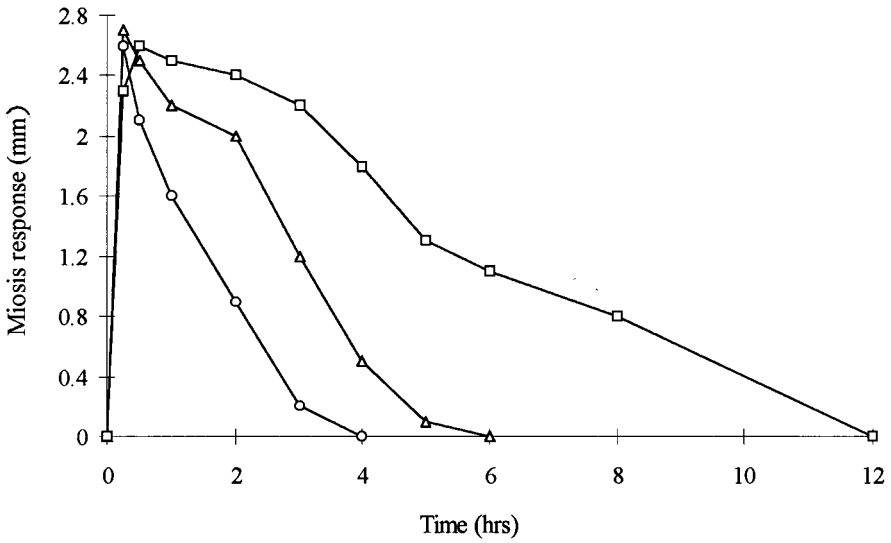

Fig. 2. Plot of miosis response (mm) as a function of time following a 5.1 mg topical instillation of pilocarpine from three different delivery systems: Pilocar® eyedrop formulation (○), Pilopine HS® gel formulation (△) and CEW device (□). All S.D. values are less than ± 10% and are omitted for clarity, ($n = 6$).

Throughout the experiment the CEW devices were well tolerated by the animals. This was evidenced by the absence of either adverse effects or physical signs of irritation such as redness and lachrimation. Also, the up and down head movements (which suggest focusing difficulty) that followed instillation of the solution and the gel were not observed with the device. It was observed that the rabbits blinked several times immediately after the instillation of the dry device. However, the blinking stopped after 1 min when the device became hydrated. Because of biodegradation or bioerosion, the CEW devices were not found in the cul-de-sac the following day. The biodegradation time was comparable to that of the simple Gelfoam® sponge. This indicates that the embedment of a retardant (CEW) in the sponge does not affect the biodegradability of the Gelfoam®.

3.1. Assessment of efficacy

The following four indicators of efficacy of pilocarpine ocular delivery systems are summarized in Table 1: (1) the peak miosis intensity (I_{max}), (2) the time to peak (T_{max}), (3) the area under the miosis response versus time curve (AUC) and (4) the duration of miosis response. The area under the miosis response versus time curve (AUC) is assumed to represent the ocular drug bioavailability from the delivery systems. The duration of miosis response is defined as the time during which a pupilliary constriction of 1 mm or greater is observed. A value of 1 mm pupilliary constriction was arbitrarily chosen as a reference point for the calculation of the duration of miosis response. I_{max}, T_{max} and duration of miosis responses were determined by linear interpolation between the data points, while AUC was calculated using the trapezoidal method.

It can be seen from Table 1 that the three pilocarpine delivery systems produced similar miosis intensity (I_{max}) values. The time to peak (T_{max}) for both the solution and gel formulations are similar (15 min), while the time to peak for the eye device is slightly longer (30 min). The maximum response produced by the device is the same as that produced by the eyedrop and is slightly lower than that produced by the gel formulation. On the basis of the AUC values, the CEW device is roughly four times more effective than the eyedrop solution and approximately twice as effective as the gel preparation. As shown also in

Table 1
Summary of the efficacy of Pilocarpine administered ocularly in different delivery systems

Ocular delivery systems	I^*_{max} (mm)	T^*_{max} (h)	AUC* (mm/h)	Duration* (h)
Eyedrop[a]	2.6	0.25	3.76	1.9
Gel[b]	2.7	0.25	7.02	3.3
CEW device	2.6	0.50	14.58	6.7

[a] Pilocar®.
[b] Pilopine HS®.
*, All S.D. values are less than $\pm$ 10%.

Table 1, the use of the CEW device results in about 4-fold improvement in the duration of miosis response over that of the eyedrop solution and a 2-fold improvement over that of the gel formulation. Undoubtedly, the CEW device is superior to the eyedrop solution and the gel in prolonging the duration of ocular pilocarpine delivery.

4. Discussion

The low efficacy of the pilocarpine eyedrop preparation is a result of two circumstances. First, upon instillation of the eyedrop a fraction of the instilled dose is lost because the fluid runs off over the lid margin and spillage. Secondly, the instilled dose that gets to the cul-de-sac is being released immediately in the lachrimal fluid and, as a consequence, is rapidly removed. An immediate spike followed by relatively short corneal contact time results in a short duration of activity.

The gel preparation which is more viscous than the eyedrop solution is somewhat better retained in the cul-de-sac, thus, allowing a longer ocular residence time. This in turn yields relatively longer duration of activity than the eyedrop solution. Nevertheless, because of the hydrophilic nature of the gel formulation, pilocarpine is released at a rapidly declining rate as the formulation is diluted by tears and sheared by blinking.

On the other hand, upon placement of the device in the cul-de-sac, and upon contact with tear fluid, the embedded drug is retained inside the intact device and is released slowly. This results in a reduced spike and a prolonged corneal contact time of pilocarpine.

Recently, the Gelfoam® sponge has been shown to be a useful vehicle for the systemic administration of relatively large peptide molecules such as insulin (Simamora et al., 1996; Lee et al., 1997) and Melanotan II (Pinsuwan et al., 1997) via the ocular route. The use of the Gelfoam® device yields substantial prolongation of insulin activity compared to the equivalent dose given as eyedrops. The duration of blood glucose lowering of insulin delivered via the device was about 10 h compared to 30 min when administered as an eyedrop. The administration of Melanotan II using a similar device yields a 67% bioavailability while the eyedrop yields only a 25% bioavailability. These results indicate that the proposed ocular insert is a useful vehicle that can be employed to deliver drug either locally to the eye or systemically upon instillation behind the lower eyelid.

In conclusion, the in-vivo results show that the CEW device produces a prolonged pilocarpine activity in rabbits when placed in the lower conjunctival sac. The application of the device produces a substantial improvement in pilocarpine efficacy over the eyedrops and the gel.

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