Mucoadhesive Microparticles in a Rapidly Dissolving Tablet for Sustained Drug Delivery to the Eye

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Purpose. To test the hypothesis that mucoadhesive microparticles formulated in a rapidly dissolving tablet can achieve sustained drug delivery to the eye.

Methods. Mucoadhesive microparticles, smaller than 5 μm were fabricated with poly(lactic-co-glycolic acid) and poly(ethylene glycol) as a core material and mucoadhesion promoter, respectively, and encapsulated pilocarpine as a model drug. These microparticles were embedded in a poly(vinyl alcohol) matrix to form a dry tablet designed to reduce rapid clearance of the microparticles on initial application to the eye.

Results. This in vitro drug release study exhibited that for all formulations, approximately 90% of pilocarpine was released during the first 10 minutes, and the remaining 10% was released slowly for 3 hours. In vivo mucoadhesion test on the rabbit eye indicated that mucoadhesive microparticles adhered significantly better to the preocular surface than other formulations. To assess the pharmacodynamics, the most prolonged pilocarpine-induced pupil constriction was observed in rabbit eyes in vivo using a tablet with mucoadhesive microparticles; it lasted up to 330 minutes.

Conclusions. The authors conclude that mucoadhesive microparticles formulated into a dry dosage form is a promising system for sustained drug delivery to the eye. (*Invest Ophthalmol Vis Sci.* 2011;52:2627–2633) DOI:10.1167/iovs.10-6465

Topical drug delivery has been widely accepted as a convenient way of drug administration to the eye. This route of administration, however, is subject to low drug bioavailability caused by rapid clearance from the preocular surface by blinking, tear drainage, and conjunctival absorption.[1,2] It has been reported that drug administration through eyedrops loses approximately 75% of the applied dose through nasolacrimal drainage almost immediately; thus, drug bioavailability in the anterior segment of the eye is <5%. The use of liquid formulation eyedrops contributes significantly to this problem because it dramatically increases preocular tear film volume, which causes spilling from the eye, increased tear drainage, and dilution of the drug concentration.[3] For these reasons, large doses, often applied according to frequent administration schedules, are required, which can cause local side effects and undesirable systemic exposure as well as low patient compliance. To resolve these problems, a drug delivery system is needed that can stay on the surface of the eye for a prolonged period and thereby increase drug bioavailability.

A variety of strategies have been suggested to increase the residence time of drug on the preocular surface. Viscous drug solutions have been proposed by using water-soluble polymers, such as carboxymethyl cellulose, hydroxypropyl cellulose, poly(vinyl pyrrolidone), and polyvinyl alcohol as additives that decrease the drug clearance rate from the preocular surface.[4–6] Although drug bioavailability has been improved to some extent, a complication is highly expected because of a rapid drug efflux from the depot through the loose polymer network. Sustained drug delivery has also been achieved by forming a drug depot at the preocular surface using gels, ointments, or ocular inserts.[7–10] However, because such systems reside on the sensitive eye surface as macroscopic depots, patient discomfort, ocular irritation, and blurred vision arise, which adversely affect patient compliance.

Therefore, a topical drug delivery system to the eye would benefit from the following conditions for improved therapeutic efficacy: prolonged residence time on the preocular surface, sustained drug delivery, and minimal eye irritation (i.e., device of small size). In this sense, biocompatible microparticles would be advantageous as a drug delivery system for the treatment of eye disorders such as glaucoma, keratoconjunctivitis sicca, or dry eye disease. Biocompatible microparticles are widely used for controlled-release drug delivery because of their ease of fabrication, simplicity of administration, and possible use in localized and targeted delivery.[11] Therapeutic agents of interest can be encapsulated in microparticles, where the drugs are released in a sustained manner by diffusion, polymer degradation, or both. Moreover, microparticles can be easily formulated into a variety of dosage forms, such as suspensions, tablets, and gels, which makes them applicable to many different localized or systemic treatments.

Because of the small size, sustained drug release, and slower preocular clearance than soluble drugs administered using liquid eyedrops, various therapeutic agents, such as dexamethasone and vancomycin, have been delivered topically to the eye by microparticles and have shown enhanced drug bioavailability.[12,13] To further increase the drug retention on the preocular surface, microparticles have also been prepared using mucoadhesive polymers, such as chitosan, pectin, hyaluronic acid, sodium carboxymethylcellulose, and polyacrylic acid.[14–19] Such microparticles adhered better to the mucus layer on the preocular surface, exhibiting enhanced drug bioavailability. However, those microparticles were applied in a liquid suspension that might still expedite tear clearance.[3]

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In this study, we proposed a dry tablet embedded with mucoadhesive microparticles as a dosage form for prolonged drug delivery to the eye. To minimize eye irritation, we designed the tablet to dissolve rapidly in tear fluid, thereby leaving only small microparticles at the eye surface. Previously, we reported that mucoadhesive microparticles, when delivered by way of a mannitol-based tablet, resided on the preocular surface longer than the other formulations tested (i.e., aqueous suspensions and tablets with non-mucoadhesive microparticles).[20,21] The prolonged residence time of microparticles was ascribed to the fact that, unlike suspensions, a rapidly dissolving tablet would not expedite tear drainage and, thus, facilitate initial contact between mucoadhesive microparticles and the preocular mucus surface.

In this study, to further hamper the clearance action of tear fluid, we used a viscosifying agent, polyvinyl alcohol, as a tablet medium instead. Microparticles were made of poly(lactic-co-glycolic acid) and poly(ethylene glycol) (PEG), which were used as the core material[13] and the mucoadhesion promoter,[22] respectively. To examine the effect of PEG, the microparticles were prepared with incorporation of three different amounts of PEG (0%, 10% and 20% wt/wt). Mucoadhesion of PEG is known to be generated by hydrogen bonds with mucins that contain hydroxyl, carboxylic acid and sulfate groups.[23] These materials are also widely established as safe for various medical applications.[24–26] The size of microparticles used in this study was well below 10 μm to avoid possible eye irritation and for safe clearance through the lacrimal canals, which are 300 to 500 μm in diameter.[2] We used pilocarpine, which has been used in the treatment of glaucoma, as a model drug in this study because the drug-induced pupil constriction could serve as a good indicator of pharmacodynamics. Thus, the drug was encapsulated in microparticles to examine the drug delivery profile in vitro and sustained drug activity in vivo. Overall, this study is novel over previous work as the first to examine mucoadhesive microparticles administered using a rapidly dissolving tablet and characterizing the resultant sustained release by measuring the pharmacodynamics of pilocarpine delivery to the rabbit eye.

Materials and Methods

Materials

Poly(lactic-co-glycolic acid) (PLG, 50:50; lot number: LP-353; MWt, 15 kDa; intrinsic viscosity, 0.15–0.25 dL/g) and polyethylene glycol (PEG; average MWt, 6 kDa) were obtained from Lakeshore Biomaterials (Birmingham, AL) and Acros Organics (Morris Plains, NJ), respectively. Polyvinyl alcohol (PVA; 87%–89% hydrolyzed, average MWt, 31–50 kDa), Nile Red, and pilocarpine were purchased from Sigma Chemical (St. Louis, MO). Methylene chloride, methanol, triethylamine, and phosphoric acid of high purity were obtained from Fisher Scientific (Pittsburgh, PA). Sodium hydroxide (50% solution) was purchased from Mallinkrodt-Baker (Phillipsburg, NJ). Hank's buffered saline solution (HBSS) was obtained from Mediatech (Manassas, VA). Proparacaine hydrochloride (0.5% ophthalmic solution) was purchased from Bausch & Lomb (Tampa, FL).

Fabrication of Microparticles Embedded in PVA Matrix

To prepare a dry tablet for in vivo study, we first prepared microparticles embedded in PVA matrix, which served as the tablet medium. Three types of microparticles were prepared in this study to examine the effect of the mucoadhesion promoter PEG. They were microparticles of PLG (PLG MP), microparticles of PLG incorporated with 10% wt/wt PEG (i.e., PLGPEG1 MP), and microparticles of PLG incorporated with 20% wt/wt PEG (i.e., PLGPEG2 MP). To prepare microparticles, a polymer solution was made by dissolving PLG or a mixture of PLG and PEG in methylene chloride. For PLG MP, 500 mg PLG was dissolved in 5 mL methylene chloride. For PLGPEG1 MP or PLGPEG2 MP, 50 mg PEG (10% wt/wt) or 100 mg PEG (20% wt/wt) was also added to the PLG solution, respectively. For in vivo mucoadhesion test of microparticles, 5 mg Nile Red was also dissolved in the polymer solution. For in vivo study of pilocarpine delivery, 0.5 mL of 10% wt/vol pilocarpine solution was added to the polymer solution and sonicated at 100 W for 2 minutes to homogeneously disperse the aqueous drug solution drops (ultrasonic converter, CV33; power supply, VC505; Sonics & Materials, Newtown, CT).

Each prepared solution was then each dispersed in 20 mL water containing 1% wt/vol PVA and sonicated at 100 W for 1 minute. The emulsion was then added to 180 mL of an aqueous solution of 1% wt/vol PVA and stirred under vacuum (approximately −20 psig) for 30 minutes to evaporate the water and methylene chloride solvents. The resultant suspension was rapidly frozen with liquid nitrogen and lyophilized for 2 days (VirTis Advantage, Gardiner, NY). In this way, the microparticles were trapped in a PVA matrix with homogeneous distribution. For in vivo study of pilocarpine delivery, the PVA matrix without microparticles was also prepared for a negative control experiment in which 5 mg pilocarpine was dissolved directly into 20 mL of 1% wt/vol PVA solution and lyophilized.

Preparation of Tablets

Four kinds of tablets were prepared using the resultant PVA matrices: tablet without microparticles, tablet containing PLG MP, tablet containing PLGPEG1 MP, and tablet containing PLGPEG2 MP. To fabricate a tablet, 20 mg PVA matrix was hand-pressed in a 3-mm diameter bore formed in a 1-cm thick acrylic sheet (Goodfellow, Oakdale, PA). In this way, each of the four tablets used for the in vivo study were prepared to contain either 500 μg pilocarpine or 50 μg Nile Red for single use.

Characterization of Microparticles

The microparticles and the tablet formulations of microparticles were imaged using a scanning electron microscope (LEO 1530; Carl Zeiss SMT, Peabody, MA). To further characterize the microparticles, the PVA matrix embedded with microparticles was dissolved in and washed with DI water to remove the PVA and collect only the microparticles. The size distribution of microparticles was determined with a Coulter counter (Multisizer 3; Beckman Coulter, Fullerton, CA) equipped with a 50-μm aperture. At least 5000 particles were counted for each sample.

In Vitro Drug Release Experiment

The in vitro release profiles of pilocarpine were examined using tablets with PLG MP, tablets with PLGPEG1 MP, and tablets with PLGPEG2 MP. Twenty milligrams of each tablet (containing 500 μg pilocarpine) was placed into 5 mL HBSS at 34°C to mimic the tear fluid at the preocular surface.[27] The supernatant was sampled at scheduled intervals for 180 minutes.

To determine the released amount of pilocarpine, the samples were assayed using an high-performance liquid chromatography (HPLC) system (1200 series; Agilent, Santa Clara, CA) with a column (Zorbax XDB-C18, 4.6 mm × 150 mm with a 5-μm particle size; Agilent). To prepare a mobile phase, 3 mL triethylamine and 13.5 mL of 85% phosphoric acid were first mixed with 983.5 mL DI water, and the pH was adjusted to 3.0 by dropwise addition of 50% sodium hydroxide solution. Then, 980 mL of the resultant solution was mixed with 20 mL methanol, giving a 1000 mL mobile phase. The flow rate of the mobile phase and the injection volume used for the HPLC assay were 1 mL/min and 50 μl, respectively. Samples were measured using an ultraviolet absorption detector operating at 214 nm at ambient temperature.

In Vivo Mucoadhesion Test

In vivo studies were performed using female New Zealand White rabbits (Myrtle's Rabbitry, Thompsons Station, TN) weighing 3.4 to 3.6

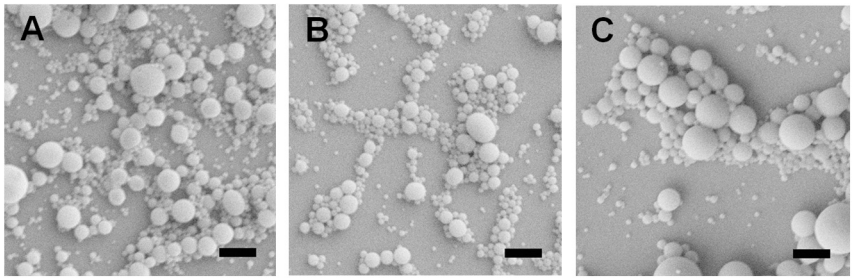

FIGURE 1. Scanning electron micrographs of pilocarpine-loaded microparticles. (**A**) PLG MP. (**B**) PLGPEG1 MP. (**C**) PLGPEG2 MP. Scale bars, 2 μm.

kg and without any known ocular defects. All experiments were conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Georgia Institute of Technology. The rabbits were housed singly in a standard cage without any restriction of food and water.

The in vivo mucoadhesion test was performed using tablets embedded with Nile Red–loaded microparticles, as previously reported.[21] Two different tablet formulations (tablet with PLG MP and tablet with PLGPEG2 MP) were tested to examine the effect of PEG on microparticle adhesion to the preocular surface. Briefly, each of the tablets (containing 50 μg Nile Red) was gently placed in the lower cul-de-sac of the rabbit eye without the use of a lid speculum, and the eyelid was closed manually for 5 minutes so the tablet could completely dissolve in the tear fluid. After administration, the rabbit was placed back in the cage and was allowed to move freely without anesthesia or sedation until samples were collected. Microparticles remaining on the preocular surface were collected 10 minutes, 30 minutes, 60 minutes, or 180 minutes after complete dissolution of the tablet. The whole preocular surface, including the cornea, the lower fornix, the upper fornix and the area close to the lacrimal caruncle, was wiped thoroughly using a cellulose surgical sponge (Ultracell Medical Technologies, North Stonington, CT) while the eye was locally anesthetized with topical administration of 25 μL of 0.5% proparacaine HCl ophthalmic solution. The surgical sponge was then submerged in acetone to completely dissolve the collected microparticles, which were assayed for Nile Red content using calibrated fluorescence spectroscopy (Photon Technology International, Birmingham, NJ). At least three eyes were tested for each tablet and each time after administration.

In Vivo Pilocarpine Delivery with Tablet Formulations

Five types of pilocarpine formulations, either tablets or solution, were tested to assess the delivery efficacy of the bioactive compound pilocarpine: pilocarpine solution, tablet without MP (i.e., PVA tablet with free pilocarpine), tablet with PLG MP, tablet with PLGPEG1 MP, and tablet with PLGPEG2 MP. Each of the tablets was placed in the lower cul-de-sac of the rabbit eye, and the eyelid was closed manually for 5 minutes so the tablet could completely dissolve in the tear fluid. For pilocarpine solution, a 50-μL drop of 1% wt/vol pilocarpine solution was applied into the lower cul-de-sac instead, where the eyelid was not forcefully closed to better mimic the administration of conventional eyedrops. Thus, the same dose of pilocarpine (500 μg) was used for tablet and solution experiments.

After administration, the eye was imaged with an infrared camera (DCR-TRV460; Sony, Tokyo, Japan) at scheduled intervals, and the longest pupil diameter was measured to assess pilocarpine-induced constriction.[28,29] The pupil constriction was calculated as a percentage of the initial pupil diameter measured before the administration of pilocarpine. In this way, the sustained drug pharmacodynamic efficacy was assessed for each of five different formulations, although the drug pharmacokinetics was not directly quantified. The experiments were performed in a dark room to avoid the effect of light on the pupil. At least three eyes were tested for each formulation.

Safety Examinations

To evaluate the safety of the tablet formulation, rabbit eyes were examined visually by a veterinarian over time after administration of tablet with PLGPEG2 MP loaded with pilocarpine. Three eyes, one from each of the three rabbits, were examined in this study.

Statistical Analysis

Mean percentages of the remaining microparticles and pupil constrictions among the different formulations were statistically analyzed using a generalized linear model ANOVA with $\alpha = 0.05$, followed by pairwise comparisons using a Tukey's post hoc test.

RESULTS

Characterization of Microparticles and Tablet Formulations

Microparticles and tablets were prepared to assess the effect of drug-loaded particles, mucoadhesion using PEG, and administration with a solid formulation tablet on pilocarpine delivery to the eye. Three different types of microparticles loaded with pilocarpine were prepared by the double emulsion (i.e., water-in-oil-in-water emulsion) method. To assess the effect of a mucoadhesion promoter, we varied the incorporated amount of PEG, giving PLG MP (0% PEG), PLGPEG1 MP (10% wt/wt PEG), and PLGPEG2 MP (20% wt/wt PEG). Regardless of the addition of PEG, all microparticles exhibited a spherical shape, as shown in Figure 1. The size of the microparticles was well below 10 μm (Figs. 1, 2), which should be appropriate for topical application to the eye without causing eye irritation.[2] For particle tracking studies, Nile Red–loaded microparticles were prepared by the single emulsion (i.e., water-in-oil emulsion) method. The morphology of these particles was similar to that of the pilocarpine-loaded microparticles (data not shown).

The tablet formulations were obtained by hand-pressing the freeze-dried PVA matrix containing microparticles. Figure 3

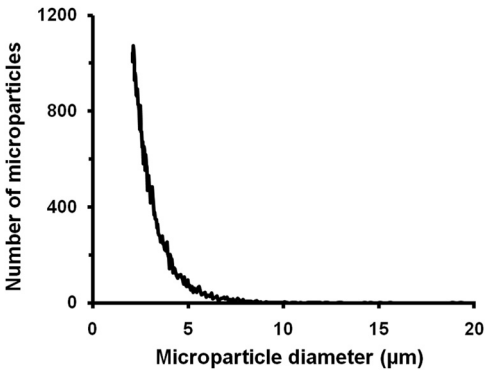

FIGURE 2. Representative size distribution of PLGPEG2 MP measured by a Coulter counter (Multisizer 3; Beckman Coulter, Fullerton, CA).

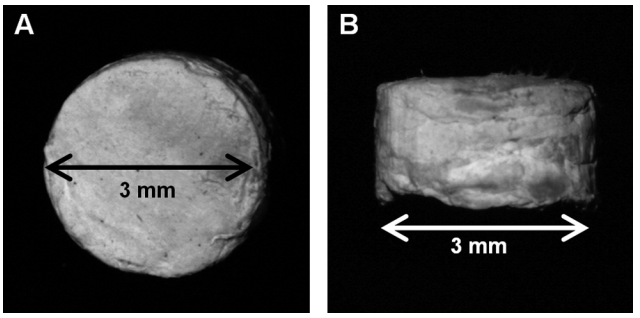

FIGURE 3. Fluorescence micrographs of a dry PVA tablet embedded with Nile Red–labeled PLGPEG2 MP. (**A**) Top view. (**B**) Side view.

shows fluorescence images of a tablet embedded with Nile Red–loaded PLGPEG2 MP. The bright signal indicates the presence of fluorescent microparticles in the tablet. The cylindrical tablet measured 3 mm in diameter and 3 mm in height, giving a volume of 21 μL, which was small enough to be administered in the lower cul-de-sac of the eye. We also examined the surface morphology of tablets without MP and tablets with PLGPEG2 MP, as shown in Figure 4. The tablet with PLGPEG2 MP clearly showed the microparticles packed in the tablet medium, whereas the tablet without MP exhibited a smooth surface composed of PVA only.

In Vitro Release Profile of Pilocarpine

Microparticles were designed to achieve an initial burst release of pilocarpine followed by subsequent sustained release. The delivery profile mimics the initial large loading dose associated with conventional eyedrop formulations combined with a sustained release for prolonged duration of action. Figure 5 shows the in vitro release profiles of pilocarpine from tablets with PLG MP, PLGPEG1 MP, or PLGPEG2 MP. A large-burst release of pilocarpine was observed with all formulations. From the same dose of 500 μg pilocarpine, >450 μg (>90%) was released during the first 10 minutes in all cases, possibly because the drug was not encapsulated in microparticles but was mostly entrapped in PVA matrix, the embedding medium of the tablet. During fabrication, much of the pilocarpine, a hydrophilic drug, would be expected to diffuse out of the PLG phase and into the aqueous PVA solution during emulsification, which was then freeze-dried altogether to produce a PVA matrix embedded with microparticles. Thus, much pilocarpine would be released rapidly through fast dissolution of this embedding medium of the tablet. On the other hand, the drug encapsulated in microparticles should be released in a sustained manner. As shown in the inset in Figure 5, the remaining pilocarpine, ~50 μg, was released slowly for 3 hours. The tablet composed of PVA only dissolved in the aqueous release media rapidly (<5 minutes), thereby having little effect on long-term drug release (data not shown).

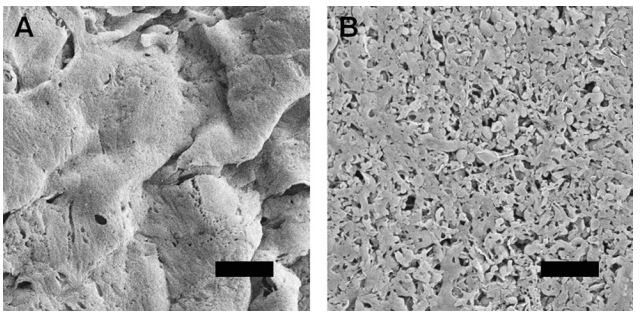

FIGURE 4. Surface morphology of the dry PVA tablets (**A**) prepared without microparticles and (**B**) embedded with PLGPEG2 MP. Images were obtained with a scanning electron microscope. Scale bar, 10 μm.

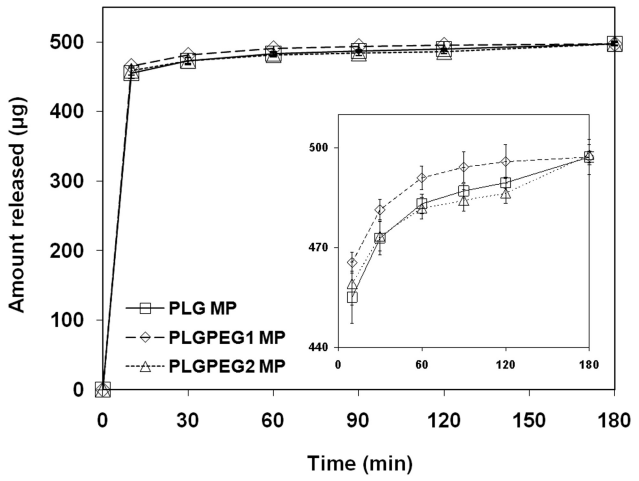

FIGURE 5. In vitro release profiles of pilocarpine from tablets embedded with PLG MP, PLGPEG1 MP, and PLGPEG2 MP. Each of the tablets was loaded with the same amount of pilocarpine (500 μg). *Inset*: detailed release profiles after the first 10 minutes. Data are presented as the average ± SD of three measurements with the same batch of each formulation.

In Vivo Mucoadhesion of Microparticles

To assess the effect of PEG on mucoadhesion property, we determined the percentage of remaining microparticles at the preocular surface over time after topical administration of tablets with PLG MP (i.e., microparticles lacking mucoadhesion) and tablets with PLGPEG2 MP (i.e., mucoadhesive microparticles) to the rabbit eye. The microparticles used for this in vivo mucoadhesion study were loaded with Nile Red to facilitate their quantitative imaging and analysis. As shown in Figure 6, PLGPEG2 MP exhibited better retention at the preocular surface than PLG MP. Especially at 10 minutes, PEG had a significant effect on the retention of microparticles ($P < 0.05$). More than 68% of microparticles remained at the eye surface when administered using the tablet with PLGPEG2 MP, whereas

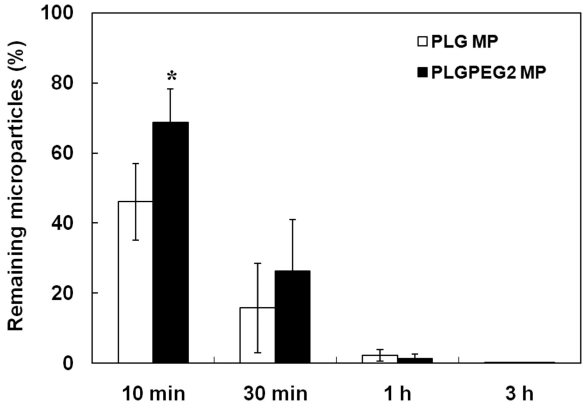

FIGURE 6. In vivo mucoadhesion of microparticles on the rabbit eye. The percentage of remaining microparticles on the preocular surface of the rabbits was measured. Almost all microparticles were cleared from the preocular surface 3 hours after administration (<0.1%). At 10 minutes, PLGPEG2 MP was significantly different from PLG MP (*$P <$ 0.05). Data are presented as the average ± SD of three measurements with the same batch of each formulation.

<50% of microparticles remained from the tablet with PLG MP. At 30 minutes, the average percentage of remaining microparticles was still larger for the tablets with PLGPEG2 MP, but this was not statistically significant ($P > 0.05$). Almost all microparticles were cleared from the preocular surface after 3 hours.

In Vivo Pilocarpine Delivery

To examine the efficacy of drug delivery to the eye, five different formulations loaded with pilocarpine were administered in the lower cul-de-sac of the eye: pilocarpine solution (50 μL solution containing 1% wt/vol pilocarpine), tablets without MP (i.e., PVA tablets with free pilocarpine), tablets with PLG MP, tablets with PLGPEG1 MP, and tablets with PLGPEG2 MP. The same dose of 500 μg pilocarpine was used for all the formulations tested in this study.

We measured pupil constrictions over time after administration of each of the formulations to assess the extent and duration of activity of the drug, as shown in Figure 7. Figure 8 shows representative images of the rabbit eyes captured with an infrared camera in the darkened room during the experiment. The initial constriction and subsequent dilation of the pupils were clearly seen during the whole procedure. The pupil constricted up to 54% to 60% for all formulations, which became most apparent 30 minutes after administration. This indicated that pilocarpine was effectively delivered to the anterior segment of the eye regardless of the type of formulation tested. This initial effect was probably due to rapid absorption of the pilocarpine that was not encapsulated in microparticles. The most rapid pupil constriction was observed with the pilocarpine solution, which had no tablet or particle to delay diffusion. The average pupil constriction was 63% at 10 minutes after administration of the pilocarpine solutions; this, however, was not significantly different from the other formulations.

From 60 minutes, the pupil started dilating with all the formulations. However, the dilation was more rapid with the formulations without mucoadhesive microparticles. The pupil diameter increased to the original size (i.e., 100%) at 180 minutes for the pilocarpine solution, the tablet without MP, and the tablet with PLG MP. This result suggested that the tablet medium itself (i.e., PVA) or the microparticles without mucoadhesion did not help prolong drug retention at the eye surface.[1]

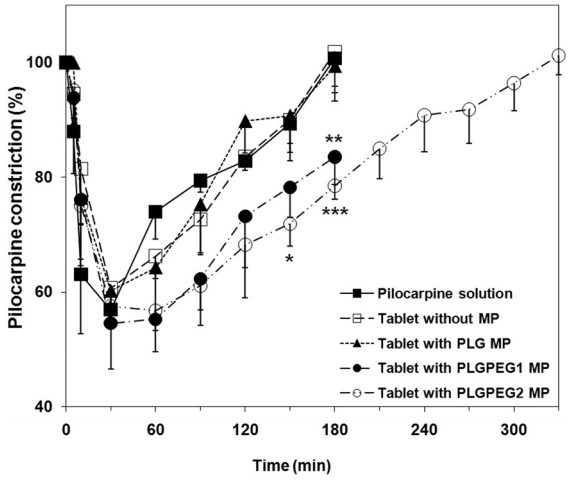

FIGURE 7. Pupil constrictions of rabbit eyes after administration of five different pilocarpine formulations. Pupil constriction was normalized to the initial pupil diameter before pilocarpine administration. The same dose of pilocarpine (500 μg) was administered in each case. At 150 minutes, the tablet with PLGPEG2 MP was significantly different from the pilocarpine solution, the tablet without MP, and the tablet with PLG MP (*$P < 0.05$). At 180 minutes, the tablet with PLGPEG1MP was significantly different from the pilocarpine solution, the tablet without MP, and the tablet with PLG MP (**$P < 0.05$). At 180 minutes, the tablet with PLGPEG2 MP was significantly different from the pilocarpine solution, the PVA tablet without MP, and the tablet with PLG MP (***$P < 0.01$). Data are presented as the average ± SD of three measurements with the same batch of each formulation.

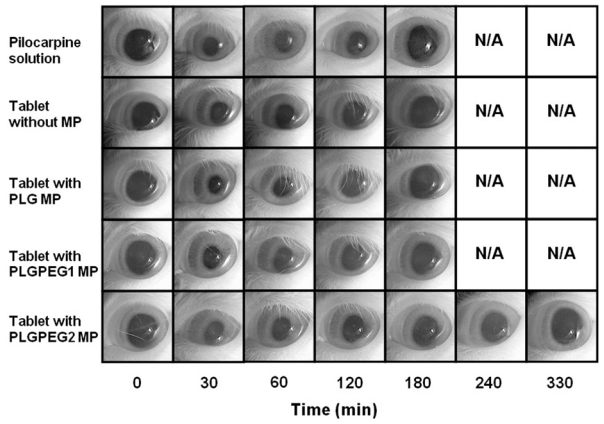

FIGURE 8. Infrared images of representative rabbit eyes after the administration of five different pilocarpine formulations. The same dose of pilocarpine (500 μg) was administered in each case.

Pupil constriction was better sustained with the formulations incorporating mucoadhesive microparticles. At 60 minutes, the tablets with PLGPEG1 MP and PLGPEG2 MP exhibited average pupil constrictions of 55% to 57%, which was not different from the maximum pupil constriction observed at 30 minutes (54%–57%). On contrary, the pupil dilated from 58%–60% to 64%–74% at 60 minutes for the other formulations without mucoadhesive microparticles. The differences became statistically significant from 150 minutes. At 150 minutes, the tablet with PLGPEG2 MP was significantly different from the pilocarpine solution, the tablet without MP, and the tablet with PLG MP ($P < 0.05$). At 180 minutes, tablets with PLGPEG1 MP and PLGPEG2 MP were significantly different from the pilocarpine solution, the tablet without MP, and the tablet with PLG MP ($P < 0.05$ and $P < 0.01$, respectively). We continuously measured pupil diameters after administration of the tablet with PLGPEG2 MP to examine the longevity of drug activity. Pupil constriction lasted for up to 330 minutes, which was an approximately two-fold increase of drug activity time compared with the pilocarpine solution, the tablet without MP, and the tablet with PLG MP.

In Vivo Safety Test

To assess the safety of the tablet formulation embedded with mucoadhesive microparticles, the rabbit eyes were examined over the course of 1 day after administration of the tablet with PLGPEG2 MP loaded with pilocarpine. Figure 9 shows representative images of the rabbit eyes that did not exhibit complications other than very mild conjunctivitis observed for up to 3 hours. This mild conjunctivitis was also observed after administration of 1 drop of pilocarpine solution (1% wt/vol) or HBSS to the rabbit eyes (data not shown).

DISCUSSION

Topical drug delivery to the eye is difficult because of the very short drug residence time on the preocular surface caused by rapid tear clearance. To resolve this and improve drug bioavailability, a drug delivery system with the following properties is

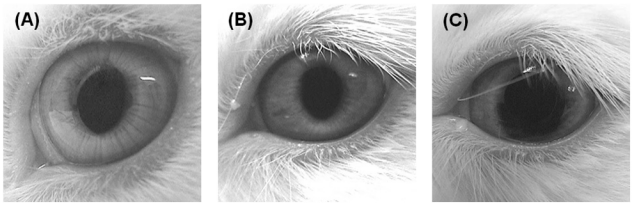

FIGURE 9. Representative images of rabbit eyes (**A**) before administration, (**B**) 3 hours after administration, and (**C**) 24 hours after administration of a tablet embedded with pilocarpine-loaded PLGPEG2 MP. The dose of pilocarpine was 500 μg. Except for very mild conjunctivitis observed for up to 3 hours, the eyes did not exhibit any other complications. The eyes were examined in bright room without control of light.

needed: prolonged residence time at the preocular surface, sustained delivery of the drug, and minimal eye irritation. In this study, therefore, we designed and assessed a rapidly dissolving tablet embedded with mucoadhesive microparticles for topical drug delivery to the eye.

Previously, we implemented a similar strategy using a mannitol-based tablet embedded with mucoadhesive microparticles in disc shape, which exhibited better retention of microparticles on the preocular surface in vivo than microparticle suspensions and tablets with non-mucoadhesive microparticles.[21] Dissolution of mannitol appeared to increase the viscosity of tear fluid to some extent, giving time for particles to interact better with the eye's topical mucous layer.

In this study, we improved the formulation design to be more suitable for delivery of a bioactive compound to the eye. Previously, the microspheres, as large as 10 μm, did not show significant improvement in mucoadhesion because of the addition of PEG.[21] In that study, the effect of PEG became apparent only when the microparticles of spherical shape were cut to form disc-shaped microparticles, which was ascribed to the increased surface-to-volume ratio of the disc-shaped microparticles with the mucous layer on the eye surface. In our current work, microparticles of smaller size ($<$5 μm) were fabricated (Figs. 1, 2) to increase the surface-to-volume ratio and thereby increase the effective microparticle surface area interacting with the mucous layer[23] and to reduce ocular irritation.[2] As a result, in vivo retention on the preocular surface was significantly improved with the presence of PEG in microparticles, even with a spherical shape (Fig. 6). Although we hypothesize that the improved retention of PEG-containing particles is due to increased mucoadhesion, an alternative explanation is that the role of PEG is to reduce particle aggregation and mucoadhesion[30] and thereby to increase the preocular residence time of the particles.

We used PVA, which has been widely accepted for ocular drug delivery formulations,[26] as the tablet medium instead of mannitol to further increase tear viscosity during tablet dissolution. Microparticles were formulated with a mucoadhesion promoter, PEG,[22] at varied incorporated amounts (i.e., 10% and 20% wt/wt PEG). Using this approach, the microparticles formulated with 20% wt/wt PEG (i.e., tablet with PLGPEG2 MP) exhibited up to a 1.5-fold increase in mucoadhesion compared with the best formulation prepared in our previous work (Fig. 6).[21]

The tablet with PLGPEG2 MP also exhibited sustained efficacy of pilocarpine in vivo (Figs. 7, 8), which was represented with the pupil constriction. The rapid drug response at the initial stage could be attributed to a large burst of pilocarpine (~90%) released primarily from the PVA matrix, as shown in the in vitro drug release profile (Fig. 5). For this reason, the maximum pupil constrictions observed at 30 minutes were not very different for all formulations (55%–60%). However, it should be noted that the pupil dilation was slower after administration of the tablet formulated with mucoadhesive microparticles, which could be attributed to the prolonged microparticle retention on the preocular surface of the tablet with PLGPEG2 MP. Although >75% of the applied dose would be lost almost instantly with pilocarpine solution,[1,2] only 31% and 74% of microparticles were removed from the preocular surface 10 minutes and 30 minutes after administration, respectively (Fig. 5). Those microparticles would release the drug for a prolonged period in the tear fluid. Of the 500-μg dose of pilocarpine, approximately 50 μg was encapsulated in the microparticles and available for sustained delivery (Fig. 5). However, this was still enough to maintain an effective drug concentration in the tear fluid, considering the small volume of tear fluid available at the preocular surface (approximately 10 μL in healthy humans)[31] and the pharmacodynamic data in Figure 7.

In some previous studies, microparticles have been used for topical ocular administration of drugs, such as dexamethasone, piroxicam, pilocarpine, and vancomycin,[12,13,18,19,32] in which the drug bioavailability was improved to a large extent compared with the drug solution. However, drug efficacy was not sustained when using microparticles of the size range acceptable for topical application. For example, although a higher peak concentration of the drug was observed in the aqueous humor compared with eyedrops of the same dose, drug elimination was faster with small microparticles (i.e., $<$10 μm), which resulted in no prolonged drug retention.[18] In some other studies, microparticles ranging in size up to 100 μm exhibited a persistent drug bioavailability for up to 180 minutes.[13] However, such large particles are expected to cause discomfort or irritation to the eye.[2] Thus, the innovation of this study is to enable sustained pharmacologic action using microparticles small enough to be expected to provide good patient acceptance. This was achieved by formulating microparticles with a mucoadhesion promoter and administering the microparticles using a tablet formulation.

As shown in previous studies, macroscopic ocular inserts could achieve good drug retention because they remain on the preocular surface for up to hours and days, slowly releasing the drug to the tear fluid.[9,33–35] However, such bulky devices applied to the ocular surface generally have lower patient acceptance.[36] In contrast, good tolerance by patients is expected with the tablet-based system presented in this study. The tablet medium dissolved rapidly in the tear fluid ($<$5 minutes), leaving only tiny microparticles smaller than 5 μm at the preocular surface.[2] Then their mucoadhesive properties enable the microparticles to attach to the eye surface and release drug for a prolonged period. After complete dissolution of the tablet within minutes, the tear viscosity should steadily decrease because of continuous dilution by tear fluid; thus, blurring is not significantly expected.

CONCLUSIONS

Low drug bioavailability is a major concern for topical drug delivery to the eye. To address this, a drug delivery system is needed that can reside on the preocular surface and release drug for a long time, thereby increasing bioavailability and sustaining drug action. In this study, we developed a dry tablet embedded with mucoadhesive microparticles as a solution. This system uses microparticles smaller than 5 μm in diameter, which is expected to reduce ocular irritation and increase patient compliance relative to larger microparticles and macroscopic ocular inserts. In addition, the microparticles incorporated a mucoadhesion promoter, PEG, to facilitate microparticle binding to the mucous layer on the ocular surface. For the

first time, the microparticles were incorporated into a dry tablet formulation to avoid the increase in preocular fluid volume and the resultant rapid clearance associated with liquid eyedrops.[3] Because it rapidly dissolves, the tablet also minimizes discomfort associated with a macroscopic device and transiently provides increased viscosity to the tear fluid, which can facilitate local retention of the microparticles, giving them time to bind to the eye's mucosal surface.

This drug delivery system was shown to release pilocarpine in vitro in a sustained manner for 3 hours after an initial burst of release within the first 10 minutes. After administration of the tablet formulation to the lower cul-de-sac of the rabbit eye in vivo, mucoadhesive microparticles adhered to the mucous layer on the preocular surface significantly longer than microparticles lacking mucoadhesive properties. Additional in vivo tests in rabbits revealed that pupil constriction by pilocarpine lasted up to 330 minutes, which was an approximately twofold increase in drug activity time compared with liquid pilocarpine eyedrops and tablets with non-mucoadhesive microparticles. Overall, we conclude that a rapidly dissolving tablet embedded with mucoadhesive microparticles is a novel method of topical drug administration that can achieve sustained drug delivery to the eye.

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