Published in final edited form as:
J Pharm Sci. 2013 February ; 102(2): . doi:10.1002/jps.23370.

Influence of Drug Lipophilicity on Drug Release from Sclera After Iontophoretic Delivery of Mixed Micellar Carrier System to Human Sclera

Poonam Chopra, **Jinsong Hao**, and **S. Kevin Li**
Division of Pharmaceutical Sciences, James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, Ohio 45267

Abstract

Mixed micelles prepared using sodium taurocholate (TA) and egg lecithin (LE) were previously found to be an effective carrier for sustained release of a poorly water-soluble drug in transscleral iontophoretic delivery. The objectives of the present study were to investigate the effects of drug lipophilicity upon micellar carrier solubilization potential and drug release profiles from the sclera after iontophoretic delivery of model lipophilic drugs dexamethasone (DEX), triamcinolone acetonide (TRIAM), and β-estradiol (E2β) with a mixed micellar carrier system of TA–LE (1:1 mole ratio). In this study, the micellar carrier system was characterized for drug solubilization. The micelles encapsulating these drugs were evaluated for transscleral passive and 2-mA iontophoretic delivery (both cathodal and anodal) and drug release from excised human sclera *in vitro*. The results show that drug solubility enhancement of the micellar carrier system increased with increasing drug lipophilicity. The more lipophilic drugs E2β and TRIAM displayed slower drug release from the sclera compared with the less lipophilic drug DEX after iontophoretic drug delivery with the mixed micelles. These results suggest that the combination of transscleral iontophoresis and micellar carriers is more effective in sustaining transscleral delivery of the more lipophilic drugs studied in this investigation.

Keywords

human sclera; lipophilic drugs; micelles; iontophoresis; sustained release; corticosteroids; ophthalmic drug delivery; nanoparticles

INTRODUCTION

Effective drug delivery to the posterior segment of the eye is vital in the treatment of posterior eye disorders such as age-related macular degeneration, diabetic retinopathy, uveitis, and choroidal neovascularization. Because of the presence of intraocular barriers, topical drug application cannot achieve therapeutic concentration at the posterior segment of the eye[1,2] and therefore intraocular injections are the main route of administration to treat these posterior eye diseases.[3–5] Although intraocular injections can provide adequate drug levels in the posterior segment of the eye, these injections are invasive with potential complications such as cataract, retinal detachment, vitreous hemorrhage, and endophthalmitis.[6,7] To overcome the risks associated with intraocular injections in the treatment of chronic posterior eye diseases, the transscleral route—with the large surface area and high sclera permeability—may offer a promising alternative,[8–12] and techniques of sustained-release drug delivery through this route are attractive. One of the noninvasive methods to enhance drug delivery to the posterior segment of the eye is transscleral iontophoresis,[13,14] but this method generally does not provide sustained-release drug delivery.

In our previous study, a mixed micellar carrier system composed of egg lecithin (LE) and taurocholate (TA) of 1:4 mole ratio was developed to enhance the aqueous solubility of dexamethasone (DEX). This study showed that the combination of micellar nanocarriers and transscleral iontophoresis could enhance drug delivery into the sclera and provide sustained release of the drug.[15] The mixed micellar system prepared with LE–TA (1:4 mole ratio) had both simple and mixed micelles coexisted in the carrier system and both these carriers are responsible for the enhancement of DEX solubilization and in the sustained release of DEX after iontophoretic delivery into and across the sclera. Because it has been reported in the literature that phospholipids (egg phophatidylcholine) play an important role in the solubilization of poorly water-soluble drug in mixed micelles and an increase in LE composition (i.e., mole ratio) in the micelles enhances the solubility of the drug in the micelles[16];mixed micellar carrier systems prepared with higher LE to TA mole ratios are believed to provide better sustained drug delivery with transscleral iontophoresis. However, the interplay of drug lipophilicity, drug solubilization, and sustained drug release of these mixed micelles in transscleral delivery is not well understood.

The objectives of the present study were to (a) determine the influence of drug lipophilicity on the solubilization potential of a mixed micellar carrier system (LE–TA 1:1, mole ratio) and (b) study the relationship between drug lipophilicity and drug release profiles of the mixed micellar carrier system. DEX, triamcinolone acetonide (TRIAM), and β-estradiol (E2β) were the model drugs selected in this study, which represented a range of lipophilicity indicated by the logarithm of octanol–water partition coefficient (log $K_{o/w}$) from 1.8 to 3.5. Table 1 summarizes the physicochemical properties of these model permeants.

MATERIALS AND METHODS

Materials

^{3}H-DEX [6,7-^{3}H(N)-; 23.1 Ci/mmol] was purchased from PerkinElmer Life and Analytical Sciences (Boston, Massachusetts) with purity of at least 97%. ^{3}H-TRIAM (0.8 Ci/mM) and ^{3}H-E2β (2,4-^{3}H; 24.5 Ci/ mmol) were purchased from Moravek Biochemicals and Radiochemicals (Brea, California). TA was purchased from Sigma–Aldrich (St. Louis, Missouri). LE (from eggs, purity >90%) was purchased from Indofine Chemical (Hillsborough, New Jersey). DEX, TRIAM, and E2β were purchased from Letco Medical (Decatur, Alabama). Phosphate-buffered saline (PBS; 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride, pH 7.4) was prepared using distilled, deionized water and PBS tablets as described by the manufacturer (Sigma–Aldrich). PBS with 2% (w/v) bovine serum albumin (BSA) was prepared by dissolving BSA (purity >98%, molecular weight 68 kDa; Sigma–Aldrich) in PBS. Filter membranes (MSE syringe filters, 0.22 μm) were purchased from Fisher Scientific (Pittsburgh, Pennsylvania). Ethanol (denatured, anhydrous ethyl alcohol) was purchased from Fischer Scientific (Rochester, New York). High-performance liquid chromatography (HPLC)-grade acetonitrile was purchased from Pharmaco-AAPER (Shelbyville, Kentucky). All materials were used as received.

Preparation of Mixed Micellar and Control Solutions

The mixed micellar solutions at LE to TA ratio of 1:1 (mole ratio) were prepared by first dissolving appropriate amounts of TA in PBS to obtain clear solutions and then adding appropriate amounts of LE to the solutions to achieve final total lipid concentration of 95 mg/mL. In the preparation of drug-loaded mixed micellar solutions, excess amounts of drugs (DEX, TRIAM, and E2β) were added to the mixed micellar solutions followed by equilibration of the solutions at 36 ± 1°C for 48–96 h. After equilibration, the mixtures were filtered through the 0.22 μm filter membranes to obtain clear solutions.

Control solutions of saturated DEX, TRIAM, and E2β were prepared by adding excess amounts of drugs in PBS followed by equilibration in a circulating waterbath at 36 ± 1°C for 48–96 h. After equilibration, the undissolved drugs were separated from the solutions by filtration using the 0.22 μm filter membranes.

Characterization of Micellar Carrier Systems

To determine the solubilities of drugs in the mixed micellar solutions and controls, aliquots of the filtered micelle and control solutions were subjected to appropriate dilution with the HPLC mobile phase. The diluted samples were then assayed for the drugs using a HPLC system. The HPLC system (Prominence; Shimadzu, Columbia, Maryland) consisted of CBM-20A system controller, LC-20AT solvent delivery module, SIL-20A autosampler, and SPD-20A UV–visible detector. The separation was performed with Microsorb C18 column (150 mm × 4.6 mm; Varian, Inc., Palo Alto, California) at room temperature. For the assays of DEX and TRIAM, a mobile phase consisting of a mixture of distilled deionized water and acetonitrile (35:65, v/v) was delivered at a flow rate of 1.0 mL/min and the drugs were detected at 284 nm wavelength. For the assay of E2β, a mobile phase consisting of a mixture of distilled deionized water and acetonitrile (50:50, v/v) was delivered at a flow rate of 1.0 mL/min, and the detection wavelength was 204 nm. Standard solutions in the appropriate concentration ranges for the drugs were prepared and used to construct the calibration curves. The samples analyzed using these calibration curves showed 2%–7% intraassay variability.

To determine the effective sizes and zeta potentials of the mixed micellar carrier systems, Malvern Zetasizer® (Nano ZS; Malvern Instruments Ltd., Worcestershire, UK) was used. The measurements from three separate batches were carried out with the mixed micellar solutions of DEX, TRIAM, and E2β in triplicate at 25°C.

Preparation of the Sclera

Human cadaver eyes were obtained from National Disease Research Interchange (NDRI, Philadelphia, Pennsylvania). The tissues were stored in moisture chambers at 4°C. Before the experiments, the sclera was soaked in PBS at room temperature and the adhering tissues on the sclera including the retina and choroid were removed with a pair of forceps. The sclera was then rinsed with PBS, cut to appropriate sizes, and equilibrated in PBS at room temperature for 30 min before its use. The use of human tissues was approved by the Institutional Review Board at the University of Cincinnati, Cincinnati, Ohio.

Transscleral Transport Study

Cadaveric human sclera was sandwiched between the two half-cells of a side-by-side diffusion cell with the choroid side facing the receptor. The diffusion cells have an effective diffusion area of 0.2 cm^2 and the temperature was maintained by a circulating water-bath at 36 ± 1°C. The volume of the donor and receptor solutions was 1.5 mL. Prior to the transport experiments, the donor solutions were prepared by adding trace amounts of radiolabeled DEX, TRIAM, and E2β (0.5–1 μCi/mL) into the DEX, TRIAM, and E2β mixed micelle or control solutions, respectively. PBS was the receptor solution in all the transport experiments. Passive transport, anodal iontophoresis, and cathodal iontophoresis experiments were performed. In the iontophoresis experiments, 2 mA direct current was applied across the sclera with a constant current iontophoretic device (Phoresor II Auto, Model PM 850; Iomed, Inc., Salt Lake City, Utah) using Ag/AgCl (cathode) and Ag (anode) as the driving electrodes. The magnitude of the current, corresponding to current density of 10 mA/cm^2, was selected based on the results of a previous study[15] and the safety limit of transscleral iontophoresis.[17] The duration of the transport experiment was 20 min. At predetermined time intervals (5, 10, 15, and 20 min), samples of 10 μL donor solution and 1

mL receptor solution were taken for assay. Fresh PBS of 1 mL was then added into the receptor to maintain a constant volume in the receptor. The samples were mixed with 10 mL of liquid scintillation cocktail (Ultima Gold™; PerkinElmer Life and Analytical Sciences, Shelton, Connecticut) and assayed by a liquid scintillation counter (LS 6500; Beckman Coulter, Fullerton, California).

To analyze the transscleral transport data, the apparent fluxes (J) were calculated from the changes in the cumulative amount (ΔQ) of the permeant transported across the sclera into the receptor chamber over time (t) divided by the effective diffusion area (A_D) for all time points using Eq. 1

$$J=\frac{1}{A_D}\frac{\Delta Q}{\Delta t} \quad (1)$$

The instantaneous fluxes at the last two time points in the transport experiments were averaged, and the apparent permeability coefficient (P) defined as the flux normalized by the donor concentration was calculated by dividing the average flux by the donor concentration (C_D) of the permeant:

$$P=\frac{1}{C_D A_D}\frac{\Delta Q}{\Delta t} \quad (2)$$

Drug Release Study

Drug release study was carried out following the transscleral transport study. Particularly, at the end of the transport experiments of DEX and TRIAM, both the donor and receptor solutions were removed, and fresh 1.5 mL of PBS was added into the receptor chamber to start the release study. A stopper was put on the donor chamber to maintain the pressure in the donor chamber. For E2β, the same drug release study method as that of DEX and TRIAM was used except that 1.5 mL of 2% (w/v) BSA in PBS was added into the receptor chamber instead of PBS. BSA was used in the receptor solution to prevent the binding of E2β to the glass diffusion cells. The duration of the drug release study was 6 days. At predetermined time intervals, 1 mL of solution was taken from the receptor for assay followed by replenishing the receptor with 1 mL fresh PBS (or 2% BSA in PBS). The samples were mixed with 10 mL of the liquid scintillation cocktail and assayed by the liquid scintillation counter. Although the results in the *in vitro* drug release study might be different from those *in vivo* because of the absence of clearance *in vitro*, the study was performed to determine the relationship between drug lipophilicity and drug release of the mixed micellar carrier system. It is believed that although the drug release profiles *in vivo* will be different from the present *in vitro* study, this will not affect the conclusion of the present study on the effect of drug lipophilicity upon drug release.

Extraction of drugs from the sclera was performed following the drug release study to determine the amounts of DEX, TRIAM, and E2β remained in the sclera at the end of the drug release study. After the last sample was taken in the drug release experiment, the sclera was removed from the side-by-side diffusion cell assembly and was immersed in 2 mL ethanol in a vial for 24 h. After 24 h of extraction, 1 mL of sample was withdrawn from the vial; mixed with 10 mL of liquid scintillation cocktail; and assayed for DEX, TRIAM, and E2β using the liquid scintillation counter. The extraction efficiency of this method was checked in a preliminary recovery study by adding known amount of the drug to the tissues followed by extracting the drug using the same method. The results showed that approximately 90% of the drug was extracted and recovered in less than 24 h. The total

amounts of DEX, TRIAM, and E2β loaded into the sclera by passive and iontophoretic delivery were calculated as the sum of the amounts of DEX, TRIAM, and E2β released from the sclera in the drug release study and the amounts of DEX, TRIAM, and E2β remained in the sclera.

Statistical Analysis

All experiments were conducted with a minimum of three replicates using sclera from different eye donors. The means ± standard deviations (SD) of the data are presented. Statistical differences were determined by Student's *t*-test. Differences were considered to be significant at a level of $p < 0.05$.

RESULTS

Characterization of Mixed Micellar Carrier Systems

Table 2 summarizes the solubilities of the drugs in the mixed micellar carrier systems. The aqueous solubilities of DEX, TRIAM, and E2β without the mixed micelles (control solutions) were 0.1, 0.02, and 0.003 mg/mL, respectively. These aqueous solubility values are essentially the same as those reported in the literature (Estimation Programs Interface Suite) (Table 1). The amounts of DEX, TRIAM, and E2β solubilized in the mixed micellar carrier systems were 0.74, 0.22, and 0.13 mg/mL, respectively. Compared with the aqueous solubilities measured in the controls, the mixed micelles significantly increased the solubilities of the drugs. Figure 1 presents the ratios of drug solubilities in the micellar carrier solutions to those in the controls. The enhancement in drug solubility of the mixed micellar carriers was from approximately 7 to 33-fold when the log $K_{o/w}$ of the drugs increased from 1.8 to 3.5.

Table 2 also presents the hydrodynamic diameters and zeta potentials of the mixed micelles under the experimental conditions in the present study. The mean hydrodynamic diameters of the mixed micelles ranged from 4.6 to 5.3 nm. The mixed micelles of DEX were smaller than those of TRIAM and E2β. The zeta potential results of the mixed micelles indicate that the micellar carriers were net negatively charged in PBS, and the absolute values of their zeta potentials were in the order of DEX > TRIAM > E2β.

Transport Study

Table 3 summarizes the apparent permeability coefficients of human sclera for DEX, TRIAM, and E2β in the passive and anodal and cathodal iontophoretic transport experiments with the mixed micellar and control solutions. In the control experiments, significantly higher iontophoretic permeability coefficients were observed during anodal iontophoresis as compared with those of passive transport ($p < 0.05$, Student's *t*-test); anodal iontophoresis enhanced the apparent permeability coefficients of DEX, TRIAM, and E2β by approximately three to four times versus passive transport. This is consistent with the effect of electroosmosis upon DEX, TRIAM, and E2β transport across the negatively charged sclera without the mixed micelles.

In general, iontophoresis enhanced the apparent permeability coefficients of DEX, TRIAM, and E2β in the mixed micellar solutions as compared with those in passive transport. In these micellar carrier experiments, cathodal iontophoresis enhanced the apparent permeability coefficients of DEX and E2β significantly ($p < 0.05$, Student's *t*-test) and by approximately twofold and fourfold, respectively, versus their passive transport. The higher cathodal iontophoresis permeability coefficients of the drugs (except TRIAM) in the mixed micellar carrier systems are consistent with the negative zeta potentials of the mixed micelles and the direct electric field effect (i.e., electrorepulsion) as a flux enhancing

mechanism of iontophoresis for the micellar carrier systems. Anodal iontophoresis enhanced the apparent permeability coefficients of DEX, TRIAM, and E2β by approximately twofold, threefold, and fivefold, respectively, versus passive transport in the presence of the mixed micelles ($p < 0.05$, Student's *t*-test). The higher anodal iontophoretic permeability coefficients of the drugs in the mixed micellar carrier systems during anodal iontophoresis compared with those of passive delivery were likely attributed to electroosmotic-enhanced transport across the negatively charged transport pathway in the sclera, consistent with the results in a previous study.[18]

The iontophoretic permeability coefficients of DEX, TRIAM, and E2β reveal no relationship between drug lipophilicity and transscleral iontophoretic delivery of the drugs in the mixed micellar carrier systems. The transport behavior of these mixed micelles does not seem to be influenced by the lipophilicities of the drugs loaded into the micelles. Iontophoretic drug delivery with the micellar carriers was likely controlled by the properties of the mixed micelles such as their sizes and charges. A comparison of the passive and iontophoresis results of DEX in the present study using 1:1 LE–TA (mole ratio) mixed micelles with those in a previous study[15] using 1:4 LE–TA (mole ratio) mixed micelles suggests that the transport properties of the 1:1 and 1:4 LE–TA mixed micellar carrier systems are similar.

Drug Release Study

Drug release from the sclera was investigated after the passive and iontophoretic transport study of DEX, TRIAM, and E2β. Figures 2a, 2b and 2c show, respectively, the cumulative amounts of DEX, TRIAM, and E2β released from the sclera after passive and iontophoretic delivery. As shown in Figures 2a–2c, drug release from the sclera in the micellar carrier experiments was enhanced after both cathodal and anodal iontophoretic delivery, and anodal iontophoresis provided better drug release enhancement than cathodal iontophoresis for TRIAM and E2β. In the control experiments without the mixed micelles, drug release was enhanced after anodal iontophoresis as compared with those after passive transport and cathodal iontophoresis, consistent with the permeability enhancement observed in the transport study.

The results in the drug extraction study of the sclera at the end of the drug release study showed that less than 12% of the total drugs loaded into the sclera remained in the tissue after the release study (except E2β, which was less than 25%). For example, the amounts of drugs extracted from the sclera at the end of the drug release study were 18.2 ± 5.2, 3.1 ± 0.4, and 3.0 ± 2.5 μg/cm^2 for DEX, TRIAM, and E2β, respectively, after anodal iontophoretic delivery with the mixed micelles (vs. 183, 194, and 41 μg/cm^2 total amounts loaded into the sclera for DEX, TRIAM, and E2β, respectively). This indicates that most of the drugs loaded into the sclera were released from the sclera over the 6-day period in the drug release study. The sclera extraction study together with the drug release study show that substantial amounts of drugs were loaded into the sclera after iontophoretic delivery compared with those after passive delivery, and drug delivery with the mixed micellar carrier systems (both iontophoretic and passive delivery) provided higher drug loading into the sclera than those of the controls without the mixed micelles.

To examine the effect of drug lipophilicity on drug release from the sclera after passive, anodal, and cathodal iontophoretic drug delivery with the mixed micelles, the cumulative amounts of drugs released at the 48-h time point as percentage of total amounts of drugs loaded into the sclera were plotted against the log $K_{o/w}$ values of the drugs in Figure 3a and compared. As shown in Figure 3a, approximately 70%–78%, 50%–73%, and 40%–53% of DEX, TRIAM, and E2β were released from the sclera 48 h after transscleral passive and iontophoretic delivery with the mixed micelles, respectively. These results suggest that the rates of drug release decreased with increasing lipophilicity of the drugs after they were

delivered into the sclera. This can be attributed to the higher partition coefficients of the more lipophilic drugs to the mixed micelles. Without the mixed micelles, greater than 80% DEX, TRIAM, and E2β were released from the sclera in the same 48-h period, again demonstrating the role of the mixed micelles in the sustained release of DEX, TRIAM, and E2β from the sclera.

To examine the effect of drug lipophilicity on drug loading into the sclera with the mixed micellar carrier systems after passive, anodal, and cathodal iontophoretic delivery, the ratios of scleral drug loading with the mixed micellar systems to the controls were plotted against the log $K_{o/w}$ values of the drugs in Figure 3b and compared. These ratios do not show any particular trend of tissue loading behavior of the drugs based on their lipophilicities. For example, after passive transport, the ratios of scleral loading of DEX, TRIAM, and E2β were not significantly different from each other ($p > 0.05$). This is consistent with the results observed in the transport study, in which the drug delivery behavior does not seem to be influenced by drug lipophilicity. However, once the drugs were delivered into the tissue, the amounts of the drugs released from the sclera were dependent on drug lipophilicity as shown in Figure 3a (slower drug release with increasing drug lipophilicity).

DISCUSSION

Effects of Drug Lipophilicity and Transscleral Delivery

Corticosteroids such as DEX and TRIAM have traditionally been used as potent anti-inflammatory agents in the treatment of ocular diseases.[19–22] Problems associated with the delivery of these drugs include their poor aqueous solubility and poor permeability across ocular tissues. E2β is more lipophilic than DEX and TRIAM, and was used as a model drug to investigate the effect of drug lipophilicity upon the drug delivery behavior of the mixed micelles in the present study. Using DEX, TRIAM, and E2β, the relationship between drug lipophilicity and the solubilization potential of the mixed micellar systems was studied. The impacts of drug lipophilicity on drug loading into the sclera and the drug release profiles of these mixed micellar carrier systems from the tissue were evaluated to understand the interplay among drug lipophilicity, drug solubilization in the mixed micelles, and release profiles of sustained drug delivery from the sclera.

Mixed micellar carriers have a hydrophobic core for drug loading, and lipophilic drugs usually have stronger interactions with this hydrophobic core than less lipophilic drugs. This attributes to the increase in the solubilization potential of the mixed micelles to the more lipophilic drugs observed in the present study. Different from drug solubilization, drug transport into/across sclera with the mixed micellar carrier systems was observed not to be impacted by the lipophilicities of the drugs in the micellar systems but the physical properties of the mixed micelles such as their charges. Similarly, the scleral drug loading results of these mixed micellar carrier systems reveal no relationship between drug loading into the sclera and drug lipophilicity. After the drugs were loaded into the sclera, a relationship between scleral drug release and drug lipophilicity was observed. The slower drug release from the sclera with increasing drug lipophilicity may be because of the stronger drug-to-micelle and/or drug-to-sclera interactions of the more lipophilic drug. Among the transscleral delivery protocols investigated, drug loading was enhanced after iontophoretic drug delivery with the mixed micelles as compared with their passive counterparts. The higher drug loading into the sclera can be attributed to enhanced drug delivery because of iontophoresis. This trend was consistent with our previous study in which improved scleral drug loading was observed after transscleral iontophoretic delivery over passive delivery,[15] suggesting transscleral iontophoretic delivery as an efficient method of drug loading into the tissue. In practice, it is expected that sustained drug delivery from the sclera *in vivo* would not last as long as those observed *in vitro*. Although sustained drug

delivery would be shorter *in vivo* compared with *in vitro*, the use of micelles could provide sustained drug delivery after iontophoresis compared with iontophoresis alone using free drug solution (control). This suggests the utility of the micellar carriers in transscleral iontophoresis to provide sustained ocular drug delivery to reduce the frequency of iontophoresis applications.

It should be noted that high-molecular-weight drugs might not follow the same trend observed between drug lipophilicity and scleral drug release observed in the present micellar carrier study. In a separate study with cyclosporine A (CysA), a highly lipophilic large molecule, and the mixed micellar carrier system, the solubility enhancement of CysA was ~35 times higher than its aqueous solubility but no CysA was detected in the receptor chamber [PBS with 2% (w/v) BSA] in the transport and drug release experiments (data not shown). This is believed to be a result of the high lipophilicity and/or high molecular weight of CysA. In another study with salicylate, a negatively charged small molecule, and the mixed micellar carrier system, although salicylate can be delivered effectively into and across the sclera with and without the mixed micelles, sustained drug release from the sclera was not observed (data not shown). This is believed to be because of the polar nature of salicylate, and hence low encapsulation efficiency into the mixed micelles. Together, these results demonstrate the limitations of the micellar carrier system as an effective system for the sustained delivery of drugs of large molecular sizes such as CysA and high polarities such as salicylate. The method of combined iontophoresis and mixed micellar carriers in the present study is useful mainly for transscleral delivery of drugs with small molecular sizes (250–500 Da) and moderate lipophilicity.

Effects of Mixed Micellar Carrier

Mixed micelles have been shown to increase the bioavailability of poorly water-soluble drugs.[16,23,24] The mixed micellar carrier system used in the present study was composed of 1:1 LE–TA (mole ratio), which was different from those of 1:4 LE–TA (mole ratio) investigated previously. Although the hydrodynamic diameter and zeta potential of the present 1:1 LE–TA mixed micelles with DEX are not significantly different from those of the previous 1:4 LE–TA DEX mixed micelles (~4.6 vs. 4.3 nm and −68.0 vs. −61.1 mV, respectively), the 1:1 LE–TA mixed micellar system has higher solubilizing capacity than that in the previous study[15]; the mixed micellar carrier system prepared using 1:1 LE–TA in the present study enhanced the solubility of DEX more than seven times as compared with its aqueous solubility, whereas the 1:4 LE–TA mixed micellar system in the previous study enhanced the solubility of DEX by only four times.[15] The approximately twofold increase in DEX solubility in the 1:1 LE–TA mixed micellar system indicates that an increase in the concentration of LE in the mixed micelles provides better solubilization of DEX in the micelles. This difference can be attributed to the increase in the volume of the hydrophobic lipid core of the micelles and/or decrease in the concentration of simple micelles in the 1:1 LE–TA mixed micellar system compared with the 1:4 LE–TA system. In spite of the higher DEX loading into the micelles, the apparent permeability coefficients of DEX were not significantly different in the 1:1 LE–TA and 1:4 LE–TA micellar carrier systems ($p > 0.05$), probably due to the similar sizes and zeta potentials of the mixed micelles in these carrier systems. On comparing the data in the present study with those in the previous study,[15] the more than twofold difference in the amounts of DEX loaded into the sclera after anodal iontophoretic transport of 1:4 LE–TA and 1:1 LE–TA mixed micellar carrier systems suggests that the 1:1 LE–TA carrier system can provide better sustained release delivery than the 1:4 LE–TA carrier system in transscleral anodal iontophoresis. The improvement in drug loading and sustained release of the drugs from the sclera after anodal iontophoresis due to the higher drug solubility in the 1:1 LE–TA mixed micellar system in transscleral

iontophoretic delivery can be an advantage over the 1:4 LE–TA mixed micellar system developed and investigated in the previous study.[15]

CONCLUSIONS

Mixed micellar systems are well recognized for their abilities to enhance the solubility and delivery of poorly water-soluble drugs. The drugs investigated in the present study were selected based on their lipophilicities. Mixed micellar carrier systems of 1:1 LE–TA were developed to enhance the solubility of these lipophilic drugs for transscleral iontophoretic delivery, and the effects of drug lipophilicity upon transscleral transport, drug release, and scleral drug loading of the mixed micellar carrier systems were studied. The following are the main findings in the present study. The present results suggest that the solubilizing potential of the mixed micellar carrier systems for the drugs increased with increasing lipophilicity of the drugs. The 1:1 LE–TA mixed micellar carrier systems have higher solubilizing potential than the previously investigated 1:4 LE–TA mixed micelles. The drugs in the 1:1 LE–TA carrier systems were efficiently transported into human sclera and their permeation into the sclera was not affected by drug lipophilicity. The scleral drug loading data indicate that iontophoresis enhanced drug loading into the sclera. The release profiles of the drugs were related to the lipophilicities of the drugs in the mixed micellar carrier systems. The release study showed prolonged scleral drug release with increasing lipophilicity of the drugs. This suggests that lipophilic drugs are better candidates to utilize these mixed micellar carrier systems as a strategy to enhance ocular iontophoretic delivery and provide sustained drug release from the sclera in transscleral delivery. It is believed that the combination of micellar nanocarriers and transscleral iontophoresis, for example, incorporation of micelle formulation in iontophoretic device, could maintain higher drug concentrations at the site of application, provide sustained release from this site to the site of drug action in the eye, and overcome the need of frequent drug dosing compared with iontophoresis alone in ocular disease treatment.

Acknowledgments

This study was supported by a grant EY 015181 from National Eye Institute (NEI) at the National Institutes of Health (NIH). The content is solely the responsibility of the authors and does not necessarily represent the official views of NEI or NIH. The authors acknowledge the use of tissues procured by the NDRI with the support of a NIH grant 5 U42 RR006042.

REFERENCES

1. Worakul N, Robinson JR. Ocular pharmacokinetics/pharmacodynamics. Eur J Pharm Biopharm. 1997; 44(1):71–83.
2. Maurice DM. Drug delivery to the posterior segment from drops. Surv Ophthalmol. 2002; 47:S41–S52. [PubMed: 12204700]
3. Geroski DH, Edelhauser HF. Drug delivery for posterior segment eye disease. Invest Ophthalmol Vis Sci. 2000; 41(5):961–964. [PubMed: 10752928]
4. Lee SS, Hughes PM, Robinson MR. Recent advances in drug delivery systems for treating ocular complications of systemic diseases. Curr Opin Ophthalmol. 2009; 20(6):511–519. [PubMed: 19667987]
5. Meyer CH, Krohne TU, Holz FG. Intraocular pharmacokinetics after a single intravitreal injection of 1.5 mg versus 3.0 mg of bevacizumab in humans. Retina. 2011; 31(9):1877–1884. [PubMed: 21738089]
6. Jager RD, Aiello LP, Patel SC, Cunningham ET. Risks of intravitreous injection: A comprehensive review. Retina. 2004; 24(5):676–698. [PubMed: 15492621]
7. Sampat KM, Garg SJ. Complications of intravitreal injections. Curr Opin Ophthalmol. 2010; 21(3): 178–183. [PubMed: 20375895]

8. Behrens-Baumann W, Martell J. Ciprofloxacin concentration in the rabbit aqueous humor and vitreous following intravenous and subconjunctival administration. Infection. 1988; 16(1):54–57. [PubMed: 3360498]
9. Clements DB, Tailor V. A study of aqueous and serum levels of ceftazidime following subconjunctival administration. Br J Ophthalmol. 1987; 71(6):433–435. [PubMed: 3304410]
10. Raghava S, Hammond M, Kompella UB. Periocular routes for retinal drug delivery. Expert Opin Drug Deliv. 2004; 1(1):99–114. [PubMed: 16296723]
11. Olsen TW, Aaberg SY, Geroski DH, Edelhauser HF. Human sclera: Thickness and surface area. Am J Ophthalmol. 1998; 125(2):237–241. [PubMed: 9467451]
12. Watsky MA, Jablonski MM, Edelhauser HF. Comparison of conjunctival and corneal surface areas in rabbit and human. Curr Eye Res. 1988; 7(5):483–486. [PubMed: 3409715]
13. Eljarrat-Binstock E, Domb AJ. Iontophoresis: A non-invasive ocular drug delivery. J Control Release. 2006; 110(3):479–489. [PubMed: 16343678]
14. Nicoli S, Ferrari G, Quarta M, Macaluso C, Santi P. In vitro transscleral iontophoresis of high molecular weight neutral compounds. Eur J Pharm Sci. 2009; 36(4–5):486–492. [PubMed: 19110056]
15. Chopra P, Hao J, Li SK. Sustained release micellar carrier systems for iontophoretic transport of dexamethasone across human sclera. J Control Release. 2012; 160(1):96–104. [PubMed: 22306336]
16. Alkan-Onyuksel H, Son K. Mixed micelles as proliposomes for the solubilization of teniposide. Pharm Res. 1992; 9(12):1556–1562. [PubMed: 1488398]
17. Parkinson TM, Ferguson E, Febbraro S, Bakhtyari A, King M, Mundasad M. Tolerance of ocular iontophoresis in healthy volunteers. J Ocul Pharmacol Ther. 2003; 19(2):145–151. [PubMed: 12804059]
18. Chopra P, Hao J, Li SK. Iontophoretic transport of charged macromolecules across human sclera. Int J Pharm. 2010; 388(1–2):107–113. [PubMed: 20045044]
19. Saraiya NV, Goldstein DA. Dexamethasone for ocular inflammation. Expert Opin Pharmacother. 2011; 12(7):1127–1131. [PubMed: 21457057]
20. Barry A, Rousseau A, Babineau LM. The penetration of steroids into the rabbit's vitreous, choroid and retina following retrobulbar injection. Can J Ophthalmol. 1969; 4(4):365–369. [PubMed: 5822457]
21. Boscia F, Furino C, Dammacco R, Ferreri P, Sborgia L, Sborgia C. Intravitreal triamcinolone acetonide in refractory pseudophakic cystoid macular edema: Functional and anatomic results. Eur J Ophthalmol. 2005; 15(1):89–95. [PubMed: 15751245]
22. Cekic O, Chang S, Tseng JJ, Barile GR, Weissman H, Del Priore LV, Schiff WM, Weiss M, Klancnik JM Jr. Intravitreal triamcinolone treatment for macular edema associated with central retinal vein occlusion and hemiretinal vein occlusion. Retina. 2005; 25(7):846–850. [PubMed: 16205562]
23. Alkan-Onyuksel H, Ramakrishnan S, Chai HB, Pezzuto JM. Amixed micellar formulation suitable for the parenteral administration of taxol. Pharm Res. 1994; 11(2):206–212. [PubMed: 7909371]
24. Hammad MA, Muller BW. Solubility and stability of lorazepam in bile salt/soya phosphatidylcholine-mixed micelles. Drug Dev Ind Pharm. 1999; 25(4):409–417. [PubMed: 10194595]

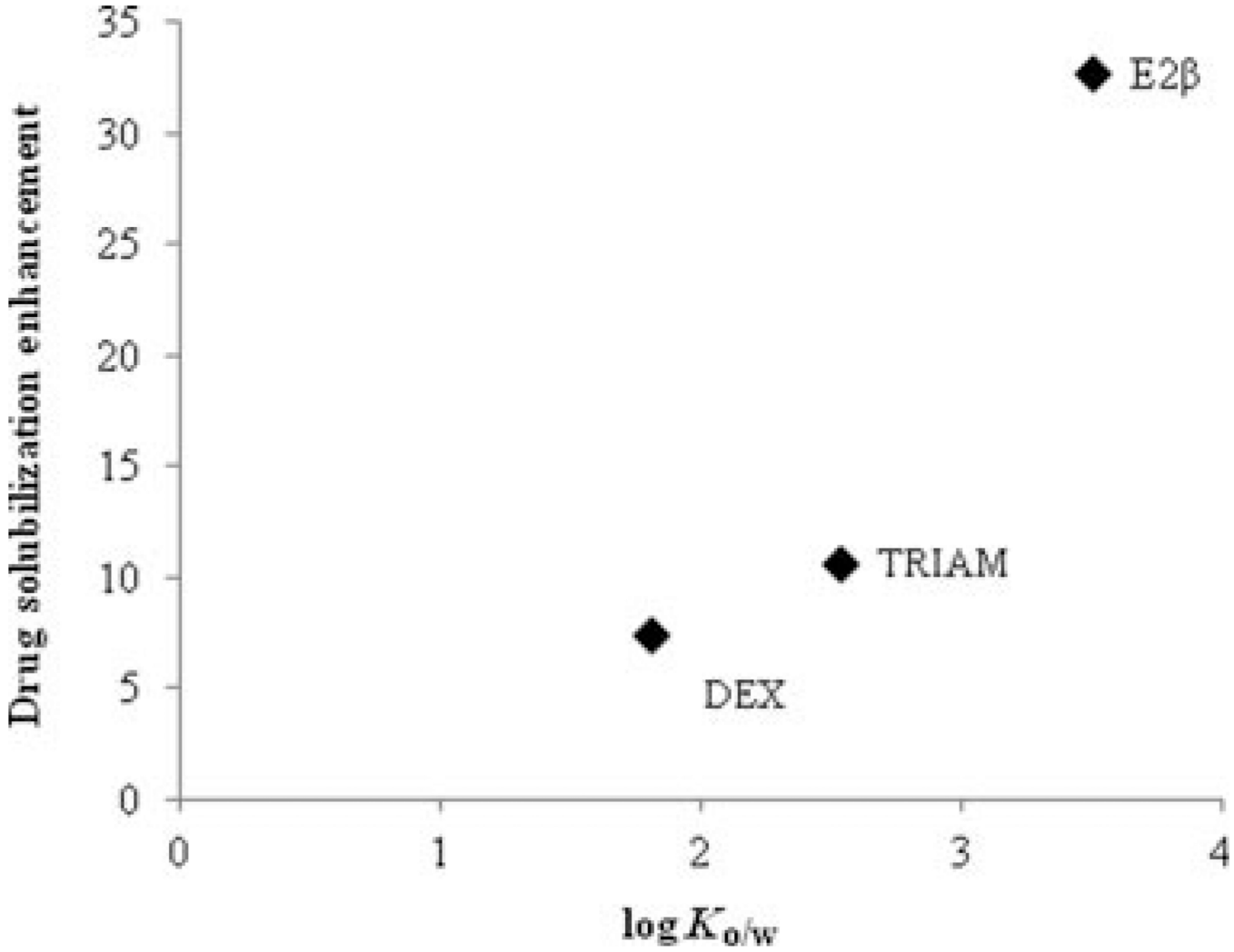

Figure 1.
Drug solubilization enhancement (ratios of solubilities of DEX, TRIAM, and E2β in the mixed micellar carrier systems to controls) versus drug log $K_{o/w}$.

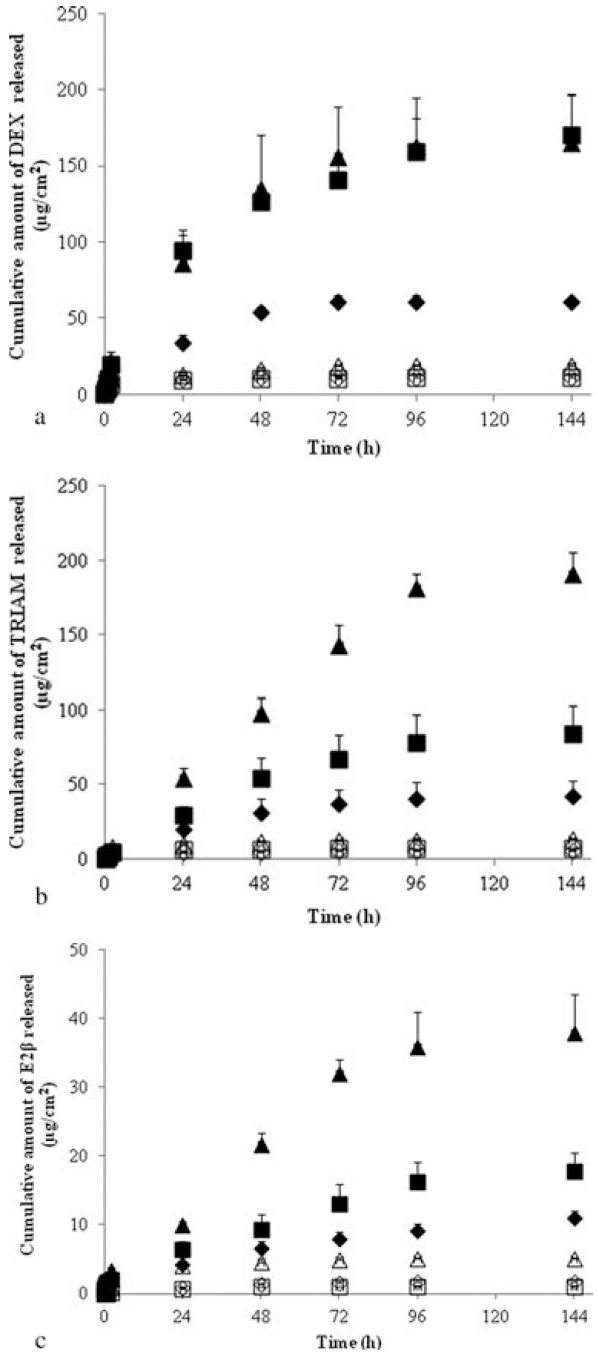

Figure 2.

Cumulative amount of (a) DEX, (b) TRIAM, and (c) E2β released from human sclera versus time in the release study performed after passive (closed diamonds), cathodal iontophoresis (closed squares), and anodal iontophoresis (closed triangles) of mixed micelles and passive (open diamonds), cathodal iontophoresis (open squares), and anodal iontophoresis (open triangles) of the controls. Data represent the mean and standard deviation, $n \geq 3$.

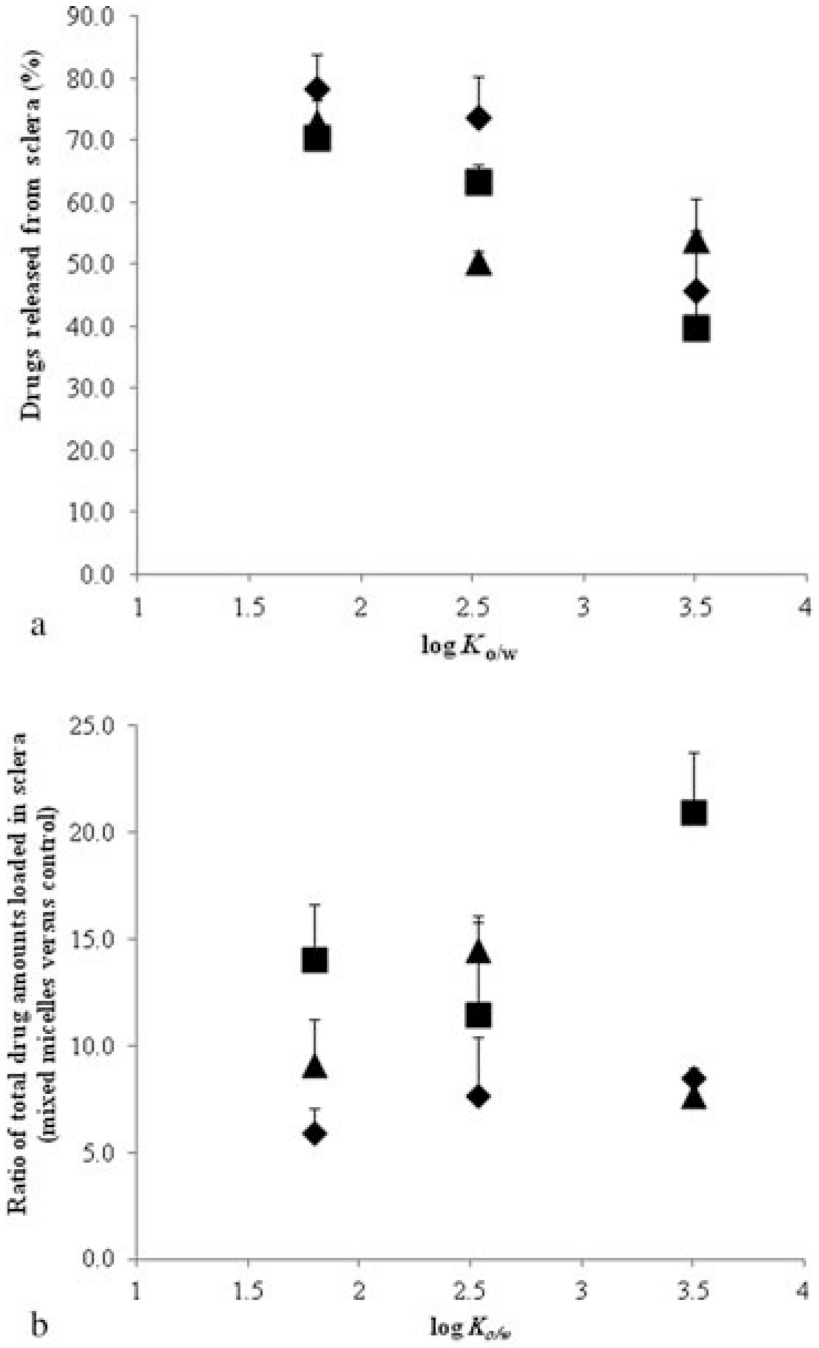

Figure 3.

(a) Percentage of DEX, TRIAM, and E2β released from sclera at time point 48 h versus drug log $K_{o/w}$ in the drug release study and (b) loading enhancement of the drugs into the sclera by the mixed micelles compared with the controls versus drug log $K_{o/w}$ after passive transport (diamonds), cathodal iontophoresis (squares), and anodal iontophoresis (triangles). Data represent the mean and standard deviation, $n \geq 3$.

Table 1

Physicochemical Properties of the Drugs used in the Present Study

Drugs	Molecular Formula	Molecular Weight (g/mol)[a]	log $K_{o/w}$ [a,b]	Aqueous Solubility (mg/mL)[a]
DEX	$C_{22}H_{29}FO_5$	392.47	1.8	0.1
TRIAM	$C_{24}H_{31}FO_6$	434.5	2.5	0.021
E2β	$C_{18}H_{24}O_2$	272.38	3.5	0.003

[a] Values obtained from the Estimation Programs Interface (EPI) database.

[b] Logarithm of octanol/water partition coefficient.

Table 2

Properties of Drug-Loaded Mixed Micellar Carrier Systems Investigated in the Present Study*

Drugs	Solubility (mg/mL)[a]	Hydrodynamic Diameter (nm)	Zeta Potential (mV)
DEX	0.74 ± 0.02	4.6 ± 0.2	−68.0 ± 1.4
TRIAM	0.22 ± 0.01	5.3 ± 0.2	−56.0 ± 1.0
E2β	0.13 ± 0.01	5.1 ± 0.3	−44.4 ± 1.3

*Mean ± SD, $n \geq 3$

[a]Solubility of drugs in the mixed micellar carrier systems at total lipid concentration of 95 mg/mL.

Table 3

Apparent Permeability Coefficients ($\times 10^{-5}$ cm/s) of DEX, TRIAM, and E2β Across Human Sclera in Passive Transport, Anodal Iontophoresis, and Cathodal Iontophoresis Experiments*

	DEX			TRIAM			E2β		
Formulations	**Passive**	**Cathodal**	**Anodal**	**Passive**	**Cathodal**	**Anodal**	**Passive**	**Cathodal**	**Anodal**
Mixed micelles	0.6 ± 0.1	1.4 ± 0.3	1.3 ± 0.2	0.24 ± 0.06	0.3 ± 0.1	0.7 ± 0.1	0.16 ± 0.04	0.7 ± 0.3	0.8 ± 0.2
Control	1.2 ± 0.6	1.2 ± 0.7	3.0 ± 1.5	1.0 ± 0.1	1.3 ± 0.2	4.0 ± 1.0	1.0 ± 0.2	1.2 ± 0.6	4.0 ± 1.7

*Mean ± SD, $n \geq 3$.