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***In Vitro* Skin Retention and Drug Permeation through Intact and Microneedle Pretreated Skin after Application of Propranolol Loaded Microemulsions**

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Abstract

Purpose—Topical beta-blockers are efficacious for treating infantile hemangiomas, but no formulations have been specifically optimized for skin delivery. Our objective was to quantify skin concentrations and drug permeation of propranolol (a nonselective beta-blocker) after application of microemulsions to intact and microneedle pretreated skin.

Methods—Four propranolol-loaded microemulsions were characterized for droplet size, surface charge, conductivity, pH, drug solubility, and drug release. Skin concentrations and drug permeation through skin were quantified using LC-MS. Skin-to-receiver ratios were used to compare the microemulsion formulations to a drug-in-PBS solution.

Results—Propranolol solubility was significantly greater in microemulsions vs PBS. Cumulative drug release from the microemulsions over 24 h ranged from 13 to 26%. Skin concentrations and drug permeation through intact skin was significantly higher from PBS; however, the skin-to-receiver ratios were significantly higher for water-rich microemulsions compared to PBS or surfactant-rich microemulsions. Microneedle pretreatment significantly increased skin concentrations for all formulations. Skin-to-receiver ratios significantly increased after microneedle pretreatment for surfactant-rich microemulsions.

Conclusions—Microemulsion formulation can be altered to elicit different drug delivery profiles through MN-treated skin. This could be advantageous for maximizing local skin drug concentrations and improving dosing schedules for infantile hemangioma treatment.

Keywords

infantile hemangioma; microemulsion; microneedle; propranolol; skin

INTRODUCTION

For skin disorders such as infantile hemangiomas (IHs), topical beta-blocker therapy can be advantageous compared to more traditional routes of administration, targeting the site of action and limiting systemic side effects (1). Propranolol and timolol are two beta-blockers currently used for the treatment of IH, though neither compound is commercially available in a preparation that has been specifically optimized for the skin. Propranolol is often compounded into a topical ointment, while timolol is delivered topically via application of prescription eye drops. Optimization of a topical beta-blocker preparation could significantly improve patient/caregiver compliance and satisfaction with therapy, as well as reduce systemic exposure and side effects in pediatric patients. Despite the advantages, topical delivery is often limited to a small number of compounds due to the restrictive nature of the stratum corneum. These compounds are typically small, lipophilic, and potent, and drugs that do not fit these physicochemical properties have limited passive diffusion to the underlying layers of the skin (2).

To increase the number of drugs available for skin delivery, various methods of enhancement have been studied. Microneedles (MNs), micron-scaled projections that create transient micropores in the stratum corneum, are one physical enhancement method to increase drug permeation to the underlying layers of the skin. MNs have gained attention in recent years for their use to increase cutaneous drug delivery for a wide range of therapeutic compounds, including small hydrophilic molecules, proteins and peptides, DNA, and nanoparticles. In addition, MNs are considered to be less painful than a hypodermic needle, and there have been no reports of skin infection after MN treatment in animals or humans (3). While MNs are typically used to increase trans-dermal delivery, various types of MNs have been used to increase local skin concentrations of many compounds (4–6).

Drug delivery through skin pretreated with solid MNs is dictated by several factors, including the extent of barrier disruption caused by the MNs, lifetime of the micropores, and the composition of the formulation applied on the treatment site. The effect of vehicle composition on MN-mediated drug delivery has been evaluated using both naltrexone (7) and diclofenac (8). For both drugs, MN pretreatment increased drug permeation across the skin compared to intact skin regardless of the vehicle used. However, drug flux through the skin was greater for formulations prepared in a hydrophilic vehicle (i.e. had a higher water content) than a more lipophilic vehicle (i.e. had a lower water content) (7,8), indicating that vehicle composition influences MN delivery and should be considered when using solid MNs.

In addition to physical methods, chemical permeation enhancers have been used to increase drug delivery into the skin for both topical and transdermal purposes. Permeation enhancers are commonly included in dermal formulations; such is the case with microemulsions. Microemulsions are composed of three distinct components: an aqueous phase, an oil phase, and a mixture of surfactants. Microemulsions are effective drug delivery vehicles for reasons including ease of preparation, good thermodynamic stability, high solubilization capacity, and enhanced drug permeation into the skin (9,10). A range of hydrophilic and hydrophobic molecules have been evaluated in microemulsions for topical indications (11–14).

The idea of combining various types of enhancement techniques is not new; synergistic effects have been observed after combining two physical methods or combining chemical enhancers with a physical method (15). However, little work has been done to evaluate drug delivery into the skin using drug-loaded microemulsions in combination with MNs. A recent study has shown that MN pretreatment increased systemic delivery of tetramethylpyrazine from a microemulsion formulation compared to microemulsion delivery through intact skin (16). This initial study shows the promise for combination treatment of microemulsions and MNs for cutaneous drug delivery. However, the potential of combining these two techniques to increase skin concentrations for topical delivery has not been explored. Furthermore, aspects of both techniques, such as microemulsion composition and MN length and number, may also influence drug delivery. It is possible that delivery of microemulsions through MN treated skin could improve local drug concentrations and decrease systemic absorption of beta-blockers used for treatment of IH.

The objective of this study was to formulate and characterize a range of microemulsion formulations for topical delivery of propranolol. The skin retention and permeation of propranolol after the application of drug-loaded microemulsion formulations was evaluated *in vitro*, and the effect of drug formulation was compared between formulations applied to intact and MN treated skin.

METHODS

Materials

(±) Propranolol hydrochloride, isopropyl alcohol, acetonitrile, ethanol, Hank's balanced salts, isopropyl myristate, methanol, metoprolol (+) tartate (+), oleic acid, Tween-80, and gentamicin sulfate were obtained from Sigma Aldrich (St. Louis, MO). Formic acid was obtained from J.T. Baker (Avanto Performance Materials, Inc., Center Valley, PA). HEPES ((4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)) and sodium bicarbonate were obtained from Research Products International (Mount Prospect, IL). Propylene glycol was obtained from Fisher Scientific (Hampton, NH). Isopropyl palmitate was obtained from Alfa Aesar (Haverhill, MA). Phosphate buffered saline (PBS) was obtained from Amresco LLC (Solon, OH).

Solubility Studies for Component Selection

Propranolol solubility was used to select the appropriate oil and co-surfactant for microemulsion formulations. An excess amount of propranolol was added to three potential oil phases (isopropyl myristate, isopropyl palmitate, and oleic acid) and three potential co-surfactants (propylene glycol, ethanol, and isopropyl alcohol). The solution was vortexed well and placed in a 37°C shaking water bath (Precision shallow form shaking water bath, ThermoScientific, Waltham, MA) at 100 rpm for 24 h. The samples were allowed to cool to room temperature and were then centrifuged at 17,000 xg (accuSpin Micro 17R, FisherScientific, Hampton, NH) for 15 min. The supernatant was appropriately diluted and analyzed by liquid chromatography-mass spectrometry (LC-MS).

Pseudo-Ternary Phase Diagram

A pseudo-ternary phase diagram was constructed to determine the area in which microemulsions exist. The oil phase (oleic acid) and the surfactant mixture (a 1:1 ratio of Tween-80 and ethanol) were added in various ratios ranging from 1:9 to 9:1 and allowed to equilibrate for 5 min under mechanical stirring. Water was titrated dropwise using a 23-gauge needle under continuous mechanical stirring until the clear solution became turbid and no small bubbles remained in the solution. The final volume of each component was converted to a percentage and plotted in a pseudo-ternary phase diagram.

Microemulsion Formulation

Based on the phase diagrams, four combinations of oil, surfactant mixture, and distilled water were selected for further analysis (Table I). The appropriate amounts of oil and surfactants were combined under magnetic stirring and allowed to equilibrate for five minutes. The appropriate amount of water was added dropwise using a 23-gauge needle under continuous stirring. The resulting formulation was allowed to stir for 10 min. For microemulsions containing propranolol (0.5% w/v), the appropriate amount of drug was added during this 10 min stirring phase.

Microemulsion Characterization

Formulation Properties—The average droplet size and polydispersity index was determined for each of the microemulsions using dynamic laser light scattering (Zetasizer Nano, Malvern Instruments, Worcestershire, UK). Conductivity and zeta potential were also measured using the Zetasizer. The pH of the microemulsion formulations was measured using a pH meter (accumet AB 150, Fisher Scientific, Hampton, NH) at 25°C.

Drug Solubility in Microemulsions—The solubility of propranolol in the microemulsion formulations, and in a control phosphate buffered saline (PBS) solution, was evaluated. Microemulsions were prepared as described, and excess amount of drug was added to the formulations. The solution was vortexed and placed in a 37°C shaking water bath (Precision shallow form shaking water bath, ThermoScientific, Waltham, MA) at 125 rpm for 48 h. The samples were allowed to cool to room temperature and were then centrifuged at 17,000 xg (accuSpin Micro 17R, FisherScientific, Hampton, NH) for 15 min. The supernatant was appropriately diluted and analyzed via LC-MS.

Drug Release from Microemulsions—The release of drug from the microemulsions was evaluated using an in-line diffusion set-up (PermeGear, Inc., Hellertown, PA). Cellulose dialysis membrane (Snakeskin® Dialysis Tubing, 10 K MWGO, ThermoScientific, Waltham, MA) was cut to the appropriate size, placed in the diffusion cell, and allowed to equilibrate with the receiver fluid (HEPES buffer with 10% ethanol, pH 7.4 modified with Hank's balanced salts and sodium bicarbonate). The receiver fluid was delivered at a flow rate of 25 μ L/min to approximate normal dermal blood flow and provide sink conditions. The receiver fluid and diffusion cells were maintained at 37°C throughout the study. The study was initiated with the addition of 200 μ L of 0.5% w/v propranolol-loaded microemulsions to the diffusion cells, and receiver solution samples were collected every hour for 24 h. The receiver samples were diluted appropriately and analyzed via LC-MS.

Diffusion Set-up Conditions and Skin Preparation

The *in vitro* permeation and skin retention of drug was evaluated using an in-line diffusion set-up (PermeGear, Inc., Hellertown, PA). Full thickness Yucatan porcine skin collected from the back (Sinclair Bio-Resources, Columbia, MO) was used as the membrane. Hair was removed with clippers, and the skin was cleaned to remove excess subcutaneous fat and stored at -20°C until the day of the study. On the day of the study the skin was thawed, cut to the appropriate size, and the thickness of each individual piece was measured. The mean $\pm$ SD thickness was 1.7 ± 0.2 mm. All studies were completed using skin from the same animal. All other conditions of the diffusion studies were the same as described above for the drug release studies.

The skin retention and drug permeation after application of four microemulsions (ME3, ME4, ME6, ME9), each containing 0.5% w/v propranolol, was evaluated for intact skin and MN treated skin ($n = 3-8$ per formulation). The control formulation was a 0.5% w/v propranolol solution in IX PBS. For the MN studies, the diffusion area of the skin was pretreated with an array containing 5 in-plane stainless steel MNs of 750 μm length, inserted five times to create a total of 25 micropores. In order to mimic the *in vivo* support of the tissue underlying the skin, the skin samples were placed on a polydimethylsiloxane polymer block during MN insertion. The skin was then mounted in the diffusion cells and charged with 200 μL of microemulsion. Studies were run for 24 h, and receiver samples were collected every three hours.

Skin Drug Concentrations

At the end of each study, the excess formulation on the surface was gently blotted off with a KimWipe®. The skin was rinsed three times with distilled water and gently blotted with a paper towel between rinses. The surface of the skin was tape stripped twice, excess skin around the diffusion area was removed, and the diffusion area was cut into nine small pieces. The weight of the skin was recorded. The skin was sonicated (1510 Ultrasonic cleaner, Branson, Danbury, CT) in methanol for 10 min, followed by two cycles of homogenization (5 min at 5 m/s) (Bead Mill 4, Fisher Scientific, Hampton, NH). Homogenized skin samples were centrifuged at 3155 xg for 30 min (Centrifuge 5804R, Eppendorf AG, Hamburg, Germany), and the supernatant was removed and centrifuged at 17,000 xg for 30 min (accuSpin Micro 17R, FisherScientific, Hampton, NH) to pellet any remaining pieces of skin. The supernatant was diluted appropriately and analyzed via LC-MS. Skin drug concentrations are reported as μmol drug/g skin.

Liquid Chromatography-Mass Spectrometry (LC-MS) Methods

Samples were separated on a Phenomenex Synergi Polar RP 80A column (C18, 4 μm , 250 mm $\times$ 2 mm, Torrance, CA) with a guard column, and the column temperature was maintained at 40°C . The mobile phase (0.075% v/v formic acid in water and acetonitrile in a 65:35 ratio) was delivered at a flow rate of 0.380 mL/min. The injection volume was 20 μL . The LC-MS system (Shimadzu, Kyoto, Japan) operated using single ion monitoring mode using atmospheric pressure chemical ionization. This was used to detect propranolol (m/z 260.30), and the internal standard metoprolol (m/z 268.30). Retention times were 5.2 min and 2.9 min, respectively. Propranolol was linear over a range of 8–2000 ng/mL.

Data Analysis

Statistical analysis was completed using GraphPad Prism 7 (GraphPad Software, Inc., Lajolla, CA). Propranolol solubility was compared between oils, co-surfactants, and microemulsions using a one-way ANOVA with Tukey's multiple comparison. The formulation properties and cumulative drug release, skin concentrations, cumulative drug permeation, and skin-to-receiver ratios from different formulations were also compared using a one-way ANOVA with Tukey's multiple comparison. A Student's *t* test with Holm-Sidak correction was used to compare skin concentrations, cumulative drug permeation, and skin-to-receiver ratios within the same formulation between intact and MN treated skin samples. A *p* value of <0.05 was considered significant.

RESULTS

Oil Phase and Co-Surfactant Selection

The solubility of propranolol in various types of oils was compared in order to select which oil to use for the microemulsions. Propranolol solubility was significantly higher in oleic acid ($269.0 \pm 52.4 \mu\text{g/mL}$) compared to isopropyl palmitate ($21.1 \pm 0.7 \mu\text{g/mL}$) or isopropyl myristate ($23.4 \pm 0.5 \mu\text{g/mL}$) ($p < 0.001$). Therefore, oleic acid was chosen as the oil phase for the microemulsions. Similarly, the solubility of propranolol in different types of co-surfactants was compared to select the co-surfactant to be used in the microemulsions. Propranolol solubility was significantly higher in ethanol ($8.3 \pm 0.3 \text{ mg/mL}$) compared to propylene glycol ($1.3 \pm 0.3 \text{ mg/mL}$; $p < 0.0001$) or isopropyl alcohol ($5.7 \pm 0.4 \text{ mg/mL}$; $p < 0.001$) and was therefore selected for future microemulsions.

Pseudo-Ternary Phase Diagram

A pseudo-ternary phase diagram was produced in order to determine the ratios in which microemulsions were formed (Fig. 1). Microemulsions were generally observed in solutions with high surfactant and low oil content. Formulations in this microemulsion formation area (shown in gray) appeared clear and flowed freely after tilting the vial at a 45° angle, while formulations outside of this area appeared turbid and were less free flowing after tilting the vial at a 45° angle. From this pseudo-ternary phase diagram, four formulations were selected to be further evaluated (Table I).

Microemulsion Properties

All of the formulations were transparent upon visual inspection. The properties of the four selected microemulsions, including droplet size, Polydispersity index, zeta potential, conductivity, and pH, are described in Table II. Droplet size of ME6 was significantly higher than the other three microemulsions ($p < 0.0001$). Additionally, the droplet size of ME4 was significantly smaller than ME3 ($p < 0.05$). Polydispersity index was significantly different only between ME3 and ME9 ($p < 0.05$). The zeta potential of ME9 was significantly higher than the others ($p < 0.05$), and the zeta potential was significantly lower for ME6 compared to ME4 ($p < 0.0001$). There was no statistically significant difference in conductivity between the four microemulsions ($p > 0.05$). Finally, the pH of ME9 was significantly lower than the other three ($p < 0.05$).

The solubility of propranolol within the microemulsions was compared to PBS (Fig. 2). Propranolol solubility was approximately twice as high or more in all of the microemulsions compared to PBS (ME3: 198.0 ± 15.6 mg/mL; ME4: 225.8 ± 14.0 mg/mL; ME6: 197.2 ± 13.9 mg/mL; ME9: 250.1 ± 9.7 mg/mL; PBS: 99.9 ± 9.2 mg/mL).

Propranolol Release from Microemulsions

Release of propranolol from the microemulsions over 24 h was evaluated using a synthetic membrane (Fig. 3). The greatest cumulative release over 24 h was observed with the ME9 formulation. However, there was no significant difference in mean cumulative drug release between the formulations (ME3: $18.6 \pm 9.6\%$; ME4: $19.1 \pm 5.7\%$; ME6: $13.1 \pm 4.1\%$; ME9: $26.8 \pm 2.1\%$; $p = 0.1218$).

Skin Retention and Permeation of Propranolol through Intact Skin

When applied to intact skin, the PBS solution provided significantly higher skin propranolol concentrations (4.82 ± 1.45 $\mu\text{mol drug/g skin}$) than any of the microemulsions ($p < 0.0001$) (Fig. 4). Of the microemulsions, ME9 produced significantly greater skin concentrations (0.040 ± 0.008 $\mu\text{mol drug/g skin}$) than ME3 (0.010 ± 0.009 $\mu\text{mol drug/g skin}$, $p < 0.05$) and ME4 (0.012 ± 0.008 $\mu\text{mol drug/g skin}$, $p < 0.05$), but not ME6 (0.020 ± 0.013 $\mu\text{mol drug/g skin}$, $p > 0.05$).

Cumulative drug permeation profiles after application of microemulsions to intact skin are shown in Fig. 5. The cumulative drug permeation into the receiver solution after 24 h was significantly greater for the PBS solution (188.6 ± 58.9 nmol) compared to any of the microemulsions ($p < 0.0001$). There was no significant difference in cumulative drug permeation between the microemulsions ($p = 0.400$), and cumulative drug permeation was below 1 nmol of propranolol for all of them (ME3: 0.767 ± 0.420 nmol; ME4: 0.630 ± 0.183 nmol; ME6: 0.603 ± 0.183 nmol; ME9: 0.397 ± 0.099 nmol).

Because the goal of topical delivery for IHs is to maximize skin concentrations while minimizing systemic delivery, skin-to-receiver ratios were compared between the formulations (Fig. 6). The mean $\pm$ SD skin-to-receiver ratio for the PBS solution was 6.04 ± 2.56 . This was similar to skin-to-receiver ratios for surfactant rich formulations (ME3: 4.20 ± 4.05 , ME4: 3.75 ± 1.73 , and ME6: 8.27 ± 6.48). The most water rich microemulsion demonstrated a higher skin-to-receiver ratio (ME9: 25.50 ± 10.69), which was significantly greater than all other microemulsions and the PBS solution ($p < 0.05$).

Skin Retention and Permeation of Propranolol through MN Treated Skin

For all formulations, skin concentrations significantly increased after MN pretreatment ($p < 0.05$, Fig. 4), and the magnitude of change between intact and MN pretreated skin varied depending on the formulation. The microemulsions produced large changes in skin concentrations after MN pretreatment, with enhancement ratios (ER) of 20.4, 17.4, 7.9, and 8.3 for ME3, ME4, ME6, and ME9 formulations, respectively. The PBS solution showed a more modest ER of 1.8 after MN pretreatment. Similar to intact skin, mean $\pm$ SD skin concentrations were significantly greater overall for the PBS solution (8.53 ± 1.46 $\mu\text{mol drug/g skin}$) compared to the microemulsion formulations ($p < 0.0001$). Between the

microemulsions, ME9 had significantly higher skin concentrations ($0.330 \pm 0.111 \mu\text{mol drug/g skin}$) than ME3 ($0.204 \pm 0.047 \mu\text{mol drug/g skin}$; $p < 0.05$), ME4 ($0.209 \pm 0.024 \mu\text{mol drug/g skin}$; $p < 0.05$), and ME6 ($0.159 \pm 0.014 \mu\text{mol drug/g skin}$; $p < 0.01$).

Cumulative drug permeation profiles from the microemulsions after application to MN treated skin are shown in Fig. 5. For all formulations, cumulative drug permeation into the receiver solution over 24 h was increased after MN pretreatment compared to intact skin, although this increase was only significant for ME9 ($p = 0.036$). Similar to what was observed with the skin concentrations, the magnitude of change in cumulative drug permeation was formulation dependent. The microemulsions showed a 3.5, 3.4, and 8.5 ER in cumulative drug permeation after MN pretreatment for ME3, ME4, and ME9, respectively. The ER for cumulative drug permeation after MN pretreatment was more modest for ME6 and PBS solution, which demonstrated a 1.2 and 1.5 ER, respectively. After MN pretreatment, mean $\pm$ SD cumulative drug permeation was significantly greater for PBS solution ($280.95 \pm 146.70 \text{ nmol}$) compared to all microemulsions ($p < 0.001$). Between the microemulsions, cumulative drug permeation was significantly lower for ME6 ($0.704 \pm 0.180 \text{ nmol}$) compared to ME3 ($2.69 \pm 1.26 \text{ nmol}$; $p < 0.05$) and ME9 ($3.37 \pm 1.25 \text{ nmol}$; $p < 0.01$), but not ME4 ($2.17 \pm 1.13 \text{ nmol}$; $p > 0.05$).

The skin-to-receiver ratios after MN pretreatment (Fig. 6) were compared to those obtained from intact skin. The skin-to-receiver ratios increased after MN pretreatment for ME3 (intact *vs* MN treated skin: 4.20 ± 4.05 *vs* 18.23 ± 6.01), ME4 (ratios for intact *vs* MN treated skin: 3.75 ± 1.73 *vs* 14.05 ± 4.60), and ME6 (ratios for intact *vs* MN treated skin: 8.27 ± 6.48 *vs* 57.90 ± 7.51), though the increase was only significant for ME3 ($p = 0.048$) and ME6 ($p = 0.005$). Alternatively, the skin-to-receiver ratios were not significantly different between intact and MN pretreated skin for the PBS solution (ratios for intact *vs* MN treated skin: 6.04 ± 2.56 *vs* 7.65 ± 4.92 ; $p = 0.610$) and ME9 (ratios for intact *vs* MN treated skin: 25.50 ± 10.69 *vs* 24.85 ± 4.71 ; $p = 0.916$).

DISCUSSION

IHs are bright red vascular lesions that affect approximately 5% of the population (17). After the unexpected discovery of propranolol for IH treatment in 2008 (18), oral beta-blocker treatment has become the mainstay of treatment for these lesions. Although effective, systemic beta-blocker therapy is associated with severe adverse events for pediatric patients, including hypoglycemia, bronchospasms, and hypotension (19). Because of this risk, beta-blocker therapy is typically limited to patients with complicated IHs, leaving many IHs untreated. Topical drug therapy would reduce the systemic side effects associated with treatment (1) and would increase the number of patients eligible for treatment. Topical beta-blockers are safe and effective (20), making them an ideal treatment option for IH patients.

Despite the clear advantages of topical delivery, there is no commercially available topical beta-blocker formulation for the skin. This is likely due to the restrictive nature of the stratum corneum, which does a remarkable job at preventing xenobiotics from entering the body. Due to the limitations imposed by the skin, various methods to enhance drug permeation are necessary for many compounds. These methods include the use of

permeation enhancers (which are commonly included in microemulsions), and MNs, which temporarily bypass the outermost layer of the skin and allow improved drug permeability (21). In the current study, the skin retention and permeation of propranolol delivered with microemulsions was evaluated through intact and MN treated skin, with the goal of improving topical beta-blocker delivery.

Microemulsion Composition

Selection of the appropriate oil phase is commonly based on the solubilization properties of the oil for the drug of interest. High solubility is necessary because a large concentration gradient between the vehicle and the skin is commonly credited as an explanation for increased drug permeation from microemulsion formulations (10). Oleic acid was chosen as the oil phase for the microemulsions in our studies for several reasons. The three oils that were evaluated differed in lipophilicity, with oleic acid being the least lipophilic and isopropyl palmitate being the most lipophilic (22,23). Montenegro *et al* demonstrated that as lipophilicity of the oil phase in microemulsions increased, drug release from the microemulsions decreased. In the current work, the compound with the lowest lipophilicity, oleic acid, was shown to have the highest solubilization capacity for propranolol. Oleic acid has also been shown to act as a permeation enhancer via disruption of the lipids within the stratum corneum (23).

The safety of microemulsions for topical delivery could be a concern due to the large surfactant content within the formulations. Tween-80, a non-ionic surfactant, was selected as the surfactant in the current study because of its ability to form microemulsions (24), its previous use for topical delivery, and its safety profile. Non-ionic surfactants cause less cutaneous irritation than ionic surfactants (25). Co-surfactants are commonly added to microemulsion formulations in order to lower the interfacial tension between the oil and aqueous phases (25). In this study, ethanol was chosen for the microemulsions due to its superior solubilization capabilities for propranolol compared to the other co-surfactants. Ethanol acts as a permeation enhancer through indirect methods by increasing the drug solubility of the compound of interest, and directly by extracting lipids from the SC (26). Ethanol is also used in a range of topical products and is generally regarded as safe for topical use (26).

In addition to selection of the components themselves, the amount of each is also important. Pseudo-ternary phase diagrams are used to establish the region in which microemulsions exist and to facilitate the selection of appropriate component ratios. We selected four formulations with low (<15%) oil content for further evaluation, as previous literature has shown this is ideal for drug delivery (27). Previous literature has also shown that surfactant and water content in microemulsion formulations are both important factors for drug delivery to the skin (27–30). Therefore, the amount of these two components was altered in order to evaluate their effects.

Microemulsion Characterization

The composition of the microemulsions can have a significant influence on the properties of the formulation, such as droplet size. Interestingly, the droplet size of ME6 was significantly

larger than the other three formulations. One possible explanation for this may be the similar amount of water and surfactants included in the formulation. A comparable trend was seen by Amarji *et al.* where microemulsions with similar amounts of surfactants and water had a higher droplet size compared to those with either higher water or surfactant content (12). Alternatively, the droplet size of this microemulsion could be the result of aggregation of the droplets (14), which may require further study.

Zeta potential describes the surface charge of the microemulsions and can indicate the long-term stability of the formulation (31). The zeta potential of ME6 was significantly lower than the other microemulsions ($p < 0.05$); this was also seen by Amarji *et al.* when characterizing a microemulsion containing similar amounts of water and surfactants (12). The slight negative charge on all of the microemulsions observed in our current study is also consistent with previous studies (11–13). High zeta potential values (± 30 mV) are typically considered ideal for stability, as formulations with values closer to zero tend to coagulate (32). Further evaluation using transmission electron microscopy (TEM) will be necessary to determine the tendency for aggregation for the microemulsions in this study.

Conductivity is used to evaluate the nature of the microemulsion formulations. Conventionally, o/w formulations have a higher conductivity value than w/o formulations (14). In our study, there was no significant difference between the conductivity values of the four formulations ($p > 0.05$). The values ranged between 2 and 5 μS , indicating that these are w/o formulations, due to their relatively low conductivity values (14).

Solubilization Capabilities of Microemulsions

In general, microemulsions tend to have increased solubilization properties compared to their neat components, potentially due to additional solubilization sites at the interfacial boundaries (29). In our study, all four microemulsions demonstrated significant increases in propranolol solubility compared to PBS. Drug delivery into the skin requires a large concentration gradient between the vehicle and the skin (2). In general, a larger concentration gradient typically increases the flux of drug through the skin. Therefore, many topical and transdermal formulations are made at or near saturation in order to take advantage of this driving force (28–30,33). In the context of the present study, this indicates that microemulsions can be formulated to contain more drug than a simple PBS solution, which may be used to achieve greater skin concentrations.

Drug Release from Microemulsions

It can be difficult to determine the release of the drug from a formulation *in vitro* when using skin as a membrane, as the skin is heterogeneous in nature with both lipophilic (stratum corneum) and aqueous (epidermis) layers (2). In order to determine propranolol release from the microemulsion formulations without the confounding effects of the stratum corneum, we conducted a release study using a homogenous membrane. The cumulative release of propranolol from the microemulsion formulations over 24 h was between 13 and 26% of the drug in the formulation, with the water rich ME9 formulation having the greatest release (Fig. 3). This is in line with other work in which water rich formulations have greater release than oil or surfactant rich microemulsions (34). The total cumulative propranolol release was

approximately 130–260 μg of propranolol, which is low compared to previous studies evaluating drug release (12,34,35). One possible explanation for this low release is the fact that the donor chamber was not occluded during the study. This was done to mimic dosing conditions used in a clinical setting for topical formulations. However, evaporation from the formulation may have occurred during the course of the *in vitro* studies, resulting in a change in composition of the donor vehicle. Within the microemulsion formulation, water and ethanol have the potential to evaporate, leaving behind the lipophilic oleic acid and Tween 80. Due to the lipophilicity of propranolol, it is possible that there was a reduction in driving force for the drug to partition out of the now lipophilic vehicle and into the hydrophilic receiver solution. The change in composition may also explain the non-linear release that was observed in this study, as previous studies have reported a linear release of drug over time (11,34,35).

Cutaneous Delivery Using Propranolol-Loaded Microemulsions

A variety of factors can influence the performance of the microemulsions (10), most of which are regarding composition. In our study, microemulsions with relatively higher water content demonstrated greater skin concentrations than microemulsions with lower water content (and higher surfactant content) for intact skin. This trend is similar to previous studies (27–29), in which microemulsion formulations with a high surfactant content exhibited reduced cutaneous delivery. Additionally, greater amounts of water in the formulation has been shown to increase drug delivery into the skin (28,33).

There are several potential explanations for these trends. First, the thermodynamic activity of the drug decreases with increasing surfactant content, reducing the driving force for drug to partition into the skin (27–30). Second, formulations with a high water content may produce a hydration effect on the stratum corneum (28,33). Water itself has been shown to increase the penetration of compounds due to fluidization of the lipid matrix (36). Finally, the mobility of the drug within the formulation can be limited in the context of a high surfactant content. Adherence of a drug to the surfactant can reduce partitioning from the formulation to the skin, limiting cutaneous delivery (29). In the present study, the thermodynamic activity of the drug is likely low for all formulations due to the low amount of drug present in the formulation. Formulations with a high surfactant content are likely to have an even lower thermodynamic activity, leading to a reduction in skin concentrations.

Skin concentrations from microemulsions were significantly lower than those obtained with a simple drug-in-PBS solution after 24 h. While this may initially seem discouraging, there are several things to consider when interpreting these data. First, the amount of propranolol loaded into the microemulsions (0.5% w/v) was low in relation to the solubility in each formulation (Fig. 2). The 0.5% w/v concentration was chosen in order to compare drug delivery to what has previously been used in a clinical setting (20). It is expected that increased drug loading into the microemulsion formulations would increase the driving force into the skin, resulting in greater skin concentrations than what was observed in this study. The influence of microemulsion solubilization on drug permeation has been described in several other studies as well (28–30,33). In the current study, it is possible that a significant driving force to partition into the skin did not exist for propranolol in the microemulsions,

potentially resulting in low skin concentrations. Optimization of the appropriate amount of drug loading into the formulations is necessary to achieve the desired balance of skin retention and drug permeation.

Second, similar to the release study, the non-occlusive conditions of the diffusion studies may have influenced microemulsion composition. Drug partitioning from a lipophilic vehicle may be limited compared to a hydrophilic vehicle (8); this, and the tendency of the lipophilic propranolol to remain in the hydrophobic vehicle, may reduce the partitioning from the microemulsion formulations into the skin, resulting in low skin concentrations.

Finally, while skin concentrations were higher with the PBS solution, the cumulative drug permeation into the receiver was also significantly higher. This is not ideal for the IH indication, as systemic beta-blocker delivery can be associated with severe adverse events, particularly in pediatric patients (19,20). The goal of topical therapy for skin disorders such as IH is to maximize the drug that is retained in the skin while minimizing the amount of drug that permeates through the skin and into systemic circulation. Therefore, we calculated the skin-to-receiver ratios of drug in order to assess the extent of drug accumulation vs drug permeation. All formulations had skin-to-receiver ratios greater than 1, indicating greater skin accumulation compared to drug permeation. The ratios ranged from 3.8 to 25.5, with formulations having a high surfactant content (ME3 and ME4) producing relatively low ratios, while the microemulsion formulation with a high water content (ME9) had a larger ratio. Baroli *et al.* observed a similar trend in which microemulsions containing larger water content demonstrated greater skin-to-receiver ratios than formulations with a larger surfactant or oil content (14). While it is clear that further optimization is necessary to increase the overall skin concentrations using microemulsions, the ability of microemulsions to produce greater skin-to-receiver ratios than a PBS solution is promising.

MN-Mediated Delivery of Propranolol from Drug Loaded Microemulsions

Skin pretreatment using solid MNs has been shown to increase the cutaneous drug delivery from various types of delivery vehicles, including nanoparticles, liposomes, and hydrogel microparticles (3). However, there is little literature regarding the use of MNs in combination with microemulsions. Zu *et al.* demonstrated that MN pretreatment in combination with drug-loaded microemulsions increased the percutaneous delivery of a hydrophilic compound both *in vitro* and *in vivo* (16). However, that study evaluated a single formulation; therefore, the effects of various formulation parameters were not considered. The current work evaluates four microemulsion formulations of various composition and the effect of composition in combination with MN pretreatment.

Skin concentrations significantly increased after MN pretreatment for all formulations evaluated (microemulsions and the PBS solution) ($p < 0.05$). However, the magnitude of increase was formulation dependent. Surfactant-rich microemulsions (ME3 and ME4) demonstrated ERs >15 , while the water-rich ME9 had an ER <10 . ME6, which had both relatively high amounts of surfactants (55%) and water (40%), had a similar change in skin concentrations as the ME9 formulation (ER 7.9). Alternatively, surfactant-rich microemulsions demonstrated smaller changes in drug permeation than the water-rich microemulsions. This trend has also been observed in binary systems of propylene glycol

and water, in which greater flux enhancement after MN pretreatment was observed for formulations with a higher water content than those with a higher propylene glycol content (7).

The effect of MN pretreatment on propranolol permeability is evident upon calculating the apparent permeability coefficient (P) using Fick's 1st law. In MN pretreated skin, P was larger compared to intact skin for all formulations. P for ME3, ME4, and ME9 (intact vs MN) were 0.346 ± 0.163 cm/h vs 1.584 ± 0.783 cm/h, 0.325 ± 0.135 cm/h vs 1.332 ± 0.694 cm/h, and 0.324 ± 0.120 cm/h vs 2.281 ± 0.834 cm/h, respectively. This resulted in ERs of 4.6, 4.1, and 7.0, respectively. In comparison, P for ME6 and PBS solution (intact vs MN) were 0.262 ± 0.015 cm/h vs 0.290 ± 0.071 cm/h, and 142.937 ± 41.692 cm/h vs 226.454 ± 117.585 cm/h, respectively. This resulted in more modest ERs of 1.1 (ME6) and 1.9 (PBS). These changes in P are similar to the trends we observed in cumulative drug permeation after MN pretreatment.

Flux through the microchannel pathway (J_{mcp}) can be calculated according to the equation:

$$J_{TOT} = J_{ISP} * f_{ISP} + J_{MCP} * f_{MCP}$$

where J_{TOT} is the mean total flux through both the intact skin and microchannel (micropore) pathways, J_{ISP} is the mean flux through the intact skin pathway, f_{ISP} is the fractional area of the intact skin pathway, J_{MCP} is the flux through the microchannel pathway, and f_{MCP} is the fractional area of the microchannel pathway. While numerous micropores are created with MN insertion, the fractional area is quite low. Therefore, f_{ISP} is approximately 1 (or 100% of the diffusion area), and $J_{ISP} * f_{ISP}$ can be simplified to J_{ISP} (7). As the fractional area of the micropores is very small, the contribution of the microchannels to the total observed flux (J_{TOT}) can be estimated by the following equation:

$$J_{MCP} = J_{TOT} - J_{ISP}$$

Using this equation, calculated J_{MCP} is 8.352 nmol/h/cm², 0.124 nmol/h/cm², 0.101 nmol/h/cm², 0.003 nmol/h/cm², and 0.196 nmol/h/cm² for PBS solution, ME3, ME4, ME6, and ME9, respectively. The microchannel pathway contributes to 78.1%, 75.6%, and 85.8% of the total flux for ME3, ME4, and ME9 respectively, while contributing 9.4% to the total flux for ME6. The pathways were more balanced for the PBS, and micropores contributed to 36.9% of total flux.

The formulation-dependent changes in skin concentrations and drug permeation influenced the skin-to-receiver ratios after MN pretreatment compared to intact skin. ME9, a water-rich formulation, showed similar changes in skin concentrations and drug permeation, resulting in skin-to-receiver ratios that were similar for MN pretreated skin and intact skin. Similarly, drug-in-PBS solution had comparable skin-to-receiver ratios for intact and MN treated skin due to a proportional increase in both skin concentrations and cumulative drug permeation. Alternatively, formulations with a high surfactant content experienced disproportional

increases in skin concentrations compared to cumulative drug permeation, resulting in a greater skin-to-receiver ratio after MN pretreatment compared to intact skin.

The increase in skin-to-receiver ratios after MN pretreatment compared to intact skin was especially apparent for the ME6 formulation (Fig. 6). There are several possible explanations for this observation. First, the ME6 formulation was unique compared to the others in that the amount of water and surfactant were relatively similar. As mentioned previously, formulations with a higher water content and lower surfactant content tend to increase the drug permeation compared to low water/high surfactant formulations for both simple binary solutions (7) and microemulsions (27,28,33). For intact skin, ME6 was in line with this trend, as the skin concentrations and skin-to-receiver ratios for this formulation fell between the high surfactant (ME3 and ME4) and high water (ME9) formulations. This trend did not hold true for the MN treated skin. This could be due to the higher viscosity noted for the ME6 formulation compared to the other microemulsion formulations. While viscosity was not formally measured in this study, it was observed that the ME6 formulation was harder to draw up in a pipet compared to the other formulations. It has previously been shown that MN-mediated delivery is influenced by the viscosity of the donor solution, with more viscous formulations producing a lower flux through MN treated skin compared to low viscosity formulations (7). Rheology measurements will need to be completed in order to confirm this hypothesis.

While the skin concentrations obtained from microemulsion formulations remained lower than those obtained with drug-in-PBS after MN pretreatment, there are several parameters that could be altered in order to achieve the ideal skin concentrations, drug permeation, and ratio between the two. MN parameters, such as length and number, may be a simple way of altering the distribution of propranolol between the skin and systemic circulation. In our study we used a low number of MNs (arrays of 5 MNs each, inserted 5 times to create 25 micropores). Other MN designs include arrays and rollers that contain more than 100 MNs, which may further enhance the drug delivery from microemulsions into the skin. Additionally, changes in microemulsion formulation can also influence cutaneous delivery. In particular, increasing the amount of drug loaded into the formulations may increase the driving force for propranolol to partition into the skin, thus potentially increasing the skin concentrations and cumulative drug permeated. In the case of IHs, which can be classified as superficial, deep, or a combination of the two (mixed IHs), it may be necessary to target different layers of the skin depending on the lesion depth. The combination of MNs and microemulsions may allow for these flexible drug delivery profiles to be achieved. Optimization of the MN and microemulsion parameters would provide further insights into the conditions necessary to achieve the desired balance of skin retention and permeation into systemic circulation.

Limitations

There are some limitations to these studies. The formulation parameters were evaluated with blank microemulsions, rather than propranolol-loaded formulations. The incorporation of a drug may influence these properties (11,13); therefore, drug-loaded characterization will be necessary going forward. The stability of the microemulsions in various storage conditions

was not formally evaluated. However, visual inspection of the formulation showed no phase separation or changes in clarity after storage at room temperature for several months. Further characterization of stability will be necessary in the future.

As the goal of topical delivery was to retain drug in the skin while minimizing drug permeation, the main parameter used in this study to compare between microemulsion formulations was the skin-to-receiver ratio. This ratio takes into account two measurements (skin concentration and cumulative drug permeated), and the variability of each measurement was incorporated into the final measurement. Thus, the overall variability for the skin-to-receiver ratios was large. Despite this, clear trends can be seen when comparing the skin-to-receiver ratios between different formulations and skin conditions.

CONCLUSIONS

Microemulsions are advantageous for topical delivery due to their solubilization capacity, ease of preparation, and thermodynamic stability. In the current study, four propranolol microemulsions of varying composition were characterized. While propranolol-loaded microemulsions did not increase the skin concentrations or drug permeation compared to a drug loaded PBS solution, microemulsions with a high water content had greater skin-to-receiver ratios, indicating their potential for topical delivery upon further optimization. The effects of MN pretreatment in combination with microemulsion delivery was formulation dependent, with a greater shift towards skin accumulation observed with low water content formulations. The ability to vary both MN and microemulsion parameters will allow for flexibility when optimizing the skin-to-receiver ratios for topical beta-blocker delivery in IH treatment.

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ABBREVIATIONS

ER	Enhancement ratio
IH	Infantile hemangioma
MN	Microneedle

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Fig. 1.

A pseudo-ternary phase diagram was constructed to determine the region in which the microemulsions exist. The gray area produced transparent microemulsion formulations, while the white area produced turbid, cloudy mixtures. From this diagram, four formulations were selected for further evaluation (noted by arrows).

Fig. 2.

Propranolol solubility in the four microemulsion formulations and a control PBS solution ($n = 3$). Propranolol solubility was significantly higher in all of the microemulsions compared to the PBS solution ($p < 0.0001$). Bars represent mean $\pm$ SD. **: $p < 0.01$; ****: $p < 0.0001$.

Fig.3. Cumulative propranolol release from the four microemulsions over 24 h: (a) ME3; (b) ME4; (c) ME6; (d) ME9. The cumulative release ranged from 13 to 26%, with the greatest release from the ME9 formulation. Points represent mean $\pm$ SD (n=3).

Fig. 4.

Skin concentrations of propranolol from a propranolol PBS solution and four microemulsion formulations for intact (black bars) and MN pretreated (white bars) skin. Skin concentrations significantly increased after MN pretreatment for all formulations. Bars represent mean $\pm$ SD ($n = 3$ for intact skin, $n = 3-8$ for MN pretreated skin). ** $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

Fig. 5. Cumulative drug permeation of propranolol from a PBS solution and four microemulsion formulations over 24 h after application to intact skin (**a**), or skin pretreated with MNs (**b**). Cumulative drug permeation after 24 h was significantly greater for the PBS solution than the microemulsions when applied to intact skin ($p < 0.0001$), though there was no significant difference in cumulative drug permeation after 24 h between the microemulsion formulations. After MN pretreatment, cumulative drug permeation significantly increased for all formulations; the PBS solution produced significantly greater cumulative concentrations compared to the microemulsions ($p < 0.001$).

Fig. 6.

Skin-to-receiver ratios of propranolol from a propranolol PBS solution and three microemulsion formulations for intact (black bars) and MN pretreated (white bars) skin ($n = 3-8$). Ratios for surfactant rich microemulsions (ME3, ME4, and ME6) increased after MN pretreatment compared to intact skin, while the PBS solution and a water rich microemulsion (ME9) produced similar ratios after MN pretreatment. Bars represent mean $\pm$ SD. *: $p < 0.05$; **: $p < 0.01$.

Table I

Composition of Microemulsion Formulations from Phase Diagrams

Formulation	Aqueous phase (%) Distilled water	Oil phase (%) Oleic acid	Surfactant mixture Tween-80: ethanol (1:1 ratio)
ME3	30	5	65
ME4	20	5	75
ME6	40	5	55
ME9	70	2	28

Table II

Formulation Properties of the Four Microemulsion Formulations Selected for Further Evaluation (Data Represented as Mean $\pm$ SD, n=3)

	Composition (% water/ % oil/ % surfactant)	Droplet size (nm)	Polydispersity index	Zeta potential (mV)	Conductivity (μ S/cm)	pH
ME3	30/5/65	33.73 $\pm$ 0.36	0.349 $\pm$ 0.008	- 12.03 $\pm$ 1.81	11.91 $\pm$ 0.09	5.55 $\pm$ 0.06
ME4	20/5/75	23.49 $\pm$ 2.00	0.305 $\pm$ 0.057	- 1 1.63 $\pm$ 1.27	4.51 $\pm$ 3.40	5.91 $\pm$ 0.1 1
ME6	40/5/55	92.54 $\pm$ 1.51	0.278 $\pm$ 0.004	-22.83 $\pm$ 0.25	1.92 $\pm$ 0.49	5.45 $\pm$ 0.11
ME9	70/2/28	28.25 $\pm$ 6.98	0.222 $\pm$ 0.056	-6.95 $\pm$ 2.53	4.00 $\pm$ 1.68	4.91 $\pm$ 0.28