Fluvastatin as a Micropore Lifetime Enhancer for Sustained Delivery Across Microneedle-Treated Skin

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Received 30 October 2013; revised 25 November 2013; accepted 6 December 2013

Published online 6 January 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23844

ABSTRACT: Microneedles (MNs), a physical skin permeation enhancement technique, facilitate drug delivery across the skin, thus enhancing the number of drugs that can be delivered transdermally in therapeutically relevant concentrations. The micropores created in the skin by MNs reseal because of normal healing processes of the skin, thus limiting the duration of the drug delivery window. Pore lifetime enhancement strategies can increase the effectiveness of MNs as a drug delivery mechanism by prolonging the delivery window. Fluvastatin (FLU), a HMGCoA reductase inhibitor, was used in this study to enhance the pore lifetime by inhibiting the synthesis of cholesterol, a major component of the stratum corneum lipids. The study showed that using FLU as a pretreatment it is possible to enhance the pore lifetime of MN-treated skin and thus allow for sustained drug delivery. The skin recovered within a 30–45-min time period following the removal of occlusion, and there was no significant irritation observed due to the treatment compared to the control sites. Thus, it can be concluded that localized skin treatment with FLU can be used to extend micropore lifetime and deliver drugs for up to 7 days across MN-treated skin.
 J Pharm Sci 103:652–660, 2014
Keywords: fluvastatin; microneedles; pore lifetime; transepidermal water loss; pharmacokinetics; transdermal drug delivery; controlled release/delivery; formulation vehicle; spectroscopy

INTRODUCTION

Transdermal drug delivery allows the delivery of drugs across the skin. For drug delivery purposes, the skin is best described as a three-layer model. From top down, these layers are stratum corneum (SC), the epidermis, and the dermis. Among these, the SC is the major rate-limiting step for the delivery of most drugs across the skin, because of its rigid structure and high lipophilicity.[1] A very small subset of molecules can effectively cross the SC barrier in therapeutically relevant amounts.[2] The structure of the SC is composed of dead keratinized remains of once rapidly dividing epidermal cells bound tightly by a lipid matrix.[2] The intercellular domain of the SC is composed of three different lipid molecules, namely, cholesterol, free fatty acids, and ceramide in equimolar ratio.[3,4] There is a plethora of literature on the proposed mechanisms of recovery after SC disruption using different methods of evaluation, such as tape stripping, acetone treatment, surfactants, etc. Formation and release of lamellar body contents is a major process in regeneration of the SC barrier following disruption or normal wear and tear. There is an initial burst (0–30 min) release of preformed lamellar body contents followed by the upregulation of lamellar body synthesis, which includes both lipid precursors and hydrolytic enzymes.[5,6] Following release at the interface of the SC and epidermis, the lamellar body contents undergo extracellular processing to form comparatively nonpolar SC lipids from their polar precursors. Thus, a host of enzymes and biochemical pathways are involved in proper functioning of the SC barrier. The modulation of a number of the precursors and the enzymes involved lead to malformation of the SC barrier following insult.[3,5] A constant molar ratio of the lipids is one of the most important parameters in barrier formation and a decrease in synthesis of any of the three lipids or their precursors leads to a delay because of malformation of lamellar bodies.[7]

Microneedles (MNs) are an alternative technique used to permeabilize the SC barrier and increase the number of drugs that can be delivered transdermally. It is a physical enhancement technique.[8] There are a number of different types of MNs and application techniques. Solid MNs are used to permeabilize the skin followed by the application of drug over the treated area, or drug is coated onto the MN itself. Polymer MNs are used to load drug into the polymer for delivery. Hollow MNs are used in conjunction with an infusion pump to facilitate the delivery of hormones and vaccines over short periods of time.[8–10] The effectiveness of MNs as a drug delivery vehicle has been established in the literature over the last decade.[11–15] The MN delivery system is very useful for short-term delivery, over a few hours.[16,17] However, the efficacy of the technique is limited for chronic therapy because of normal healing processes of the skin, which lead to resealing of the micropores anywhere between a 48–72-h timeframe under occlusion.[18–21] Thus, drug can only be delivered across MN-treated skin for a maximum of 3 days under occlusion.[15,22] Lifetime of the micropore when exposed to air is much shorter and ranges from 15 min to a few hours depending on the MN geometry, animal model, and detection method used for evaluation.[18–20] The short time frame of micropore resealing can be explained in terms of enhanced transepidermal water loss (TEWL) in the absence of occlusion. TEWL is the

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Journal of Pharmaceutical Sciences, Vol. 103, 652–660 (2014)

most important signal for barrier recovery and lamellar body secretion following insult to the skin.[23] Decreased TEWL under occlusion slows down the recovery processes of the skin.

Biochemical enhancers/lipid biosynthesis inhibitors prevent the synthesis of the essential lipids required for lamellar body synthesis and thus the proper formation of the SC. Local concentrations of specific inhibitors of the three lipid synthesis pathways, namely, cholesterol, fatty acids, and ceramides, can be used to alter the molar ratio and thus delay barrier recovery.[4] The inhibitors can be used either as a pretreatment or in the formulation. Some of the inhibitors that can be used for the above mechanism are 5-(tetradecycloxy)-2-furancarboxylic acid (TOFA) for fatty acid synthesis, fluvastatin (FLU) for cholesterol synthesis, and β-chloroalanine (BCA) for ceramide synthesis.[4]

The goal of the current study was to evaluate the effectiveness of FLU, an HMGCoA reductase (an important enzyme of the cholesterol synthesis pathway) inhibitor, as a pore lifetime enhancement agent for sustained drug delivery across MN-treated skin. Solid stainless steel MNs were used to permeabilize the skin. This technique is also known as the "poke (press) and patch" approach and is advantageous for sustained delivery because the MN is only used to permeabilize the skin and does not remain in contact with skin thereafter.[8] The current efforts were directed toward enhancing the drug delivery window to 7 days, the ideal transdermal patch wear time, by using a biochemical enhancement technique in addition to MN.

The model compound for the drug delivery project was naltrexone (NTX). It is a μ-opioid receptor antagonist used for alcohol and opioid addiction treatment. The currently approved dosage forms include an oral and an extended-release intramuscular injection.[24,25] The oral dosage form has issues with variable bioavailability and compliance in its treatment population, because of daily dosing and side effects.[24,26] The extended-release intramuscular injection is difficult to remove if there is a need for emergency opiate treatment, and also leads to serious injection site reactions.[25] Thus, NTX is a suitable candidate for transdermal patch development and an active delivery system in the form of MNs was used in this project, as the drug cannot be delivered in therapeutic concentrations via passive transdermal delivery.[15] It has been previously shown that by using MNs, NTX can be delivered at therapeutic levels for 2–3 days in humans.[15]

EXPERIMENTAL SECTION

Materials

Naltrexone HCl was purchased from Mallinckrodt (St. Louis, Missouri), and FLU sodium was purchased from Cayman chemical (Ann Arbor, Michigan). Propylene glycol (PG) and ethanol (200 proof) were purchased from Sigma (St. Louis, Missouri). Acetic acid, ammonium acetate, and benzyl alcohol were obtained from Fisher Scientific (Fair Lawn, New Jersey). 1-Octanesulfonate, sodium salt was obtained from Regis Technologies, Inc (Morton Grove, Illinois). Trifluoroacetic acid (TFA), triethylamine (TEA), methanol, ethyl acetate, and acetonitrile (ACN) were obtained from EMD chemicals (Gibbstown, New Jersey). Natrosol® (hydroxyethylcellulose) was obtained from Ashland (Wilmington, Delaware). Ethanol (70%) was obtained from Ricca chemical (Arlington, Texas). Sterile water for injection was obtained from Hospira (Lake Forest, Illinois) and water was purified using a NANOpure DIamond™, Barnstead water filtration system for all *in vitro* experiments.

High-Pressure Liquid Chromatography Methods

Naltrexone and FLU from *in vitro* studies were quantified using high-pressure liquid chromatography (HPLC). The HPLC system consisted of a Waters 717 plus auto-sampler, a Waters 600 quaternary pump, and a Waters 2487 dual-wavelength absorbance detector with Waters Empower™ software (Milford,MA). A Perkin Elmer Brownlee™ Spheri 5 VL C18 column (5 μm, 220 × 4.6 mm^2) and a C18 guard column (15 × 3.2 mm^2) were used with the UV detector set at a wavelength of 280 nm for NTX and 305 nm for FLU. The mobile phase consisted of 65:35 (v/v) ACN: (0.1% TFA with 0.065% 1-octane sulfonic acid sodium salt, adjusted to pH 3.0 with TEA aqueous phase). Samples were run at a flow rate of 1.5 mL/min. The injection volume used was 100 μL for all samples.

In Vitro Experiments

The *in vitro* studies were carried out to look at the flux across MN-treated skin and the skin concentrations of NTX and FLU in the skin. Full thickness Yucatan miniature pig skin was used for all *in vitro* experiments. All pig tissue harvesting experiments were carried out under IACUC approved protocols at the University of Kentucky. Fresh skin was cleaned to remove the excess subcutaneous fat, dermatomed and stored at −20°C. Skin was thawed and cut into small square pieces on the day of the diffusion experiment. The thickness was measured for each individual piece of skin and the average thickness of all treatment groups was maintained between 1.4 and 1.8 mm. Skin was next treated with a 5 MN in-plane array, 10 times in one direction and 10 times in a mutually perpendicular direction to generate a total of 100 nonoverlapping MN insertions within the active treatment area of 0.95 cm^2. Each MN (triangular in shape) was 750 μm long, 200 μm wide (at the base) and 75 μm thick (thickness of metal used) and the inter needle spacing was 1.35 μm. Interindividual variability for MN application was not evaluated during the current study, and all treatments were carried out by the same investigator. The pressure associated with MN application was kept consistent throughout the studies. Five treatment groups were used based on the four different vehicles used for FLU and a control for NTX only; all studies were performed in triplicate. Saturated NTX gel was prepared by mixing 90 mg/mL of NTX HCl (saturated solution) with PG (10%). A 3% hydroxyethylcellulose (HEC) gel of the NTX solution was used for the *in vitro* studies. The four different FLU treatments were FLU in 200 proof ethanol, acetone, 7:3 PG–ethanol, and 1:2:1 PG–ethanol–water. All the vehicles for FLU were based on previous studies looking at the recovery of the skin or commonly used drug deposition methods.[4,27] The concentration of FLU was 1.5% for all the formulations and 40 μL of the formulation was applied to each cell. The receiver solution was water alkalified to pH 7.4 containing 20% ethanol at 37°C. The temperature of the diffusion cell skin surface was maintained at 32°C. Samples were collected every 6 h for 48 h. All samples were analyzed using HPLC. The steady state flux of NTX was calculated using the steady-state portion of the cumulative amount permeated versus time plot. The skin concentrations of both drugs were also determined at the end of the study by extracting the drug overnight into 10 mL of ACN,