International Journal of Pharmaceutics 472 (2014) 362–368

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Vehicle influence on permeation through intact and compromised skin

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ARTICLE INFO

Article history:
Received 23 January 2014
Received in revised form 5 June 2014
Accepted 25 June 2014
Available online 27 June 2014

Keywords:
Transdermal
Percutaneous
Vehicle
Compromised skin
Diclofenac

ABSTRACT

The purpose of this study was to compare the transdermal permeation of a model compound, diclofenac diethylamine, from a hydrophilic and lipophilic vehicle across *in vitro* models simulating compromised skin. Mineral oil served as a lipophilic vehicle while 10 mM phosphate buffered saline served as a hydrophilic vehicle. Compromised skin was simulated by tape stripping, delipidization, or microneedle application and compared with intact skin as a control. Transepidermal water loss was measured to assess barrier function. Skin compromised with tape stripping and delipidization significantly ($p < 0.05$) increased permeation of diclofenac diethylamine compared to intact and microneedle treated skin with phosphate buffered saline vehicle. A similar trend in permeation was observed with mineral oil as the vehicle. For both vehicles, permeation across skin increased in the same order and correlated with degree of barrier impairment as indicated by transepidermal water loss values: intact < microneedles < tape stripping < delipidization. A study with hairless rats comparing both vehicles found the same trend, with hydrophilic vehicle having greater delivery. In conclusion, phosphate buffered saline vehicle resulted in higher permeation into and across skin compared to mineral oil vehicle for all simulated models of compromised skin.

1. Introduction

Skin is a vital organ which serves as a physical barrier between host and environment, allowing for minimal passage of water and chemicals. The barrier function is maintained by the stratum corneum, a structure rich in lipids such as ceramides, cholesterol, and free fatty acids (Jungersted et al., 2008). Measurement of transepidermal water loss (TEWL) can be used to assess skin barrier integrity. Studies have shown a change in TEWL associated with pathological skin conditions. For example, Lee et al. observed an increase in TEWL values for patients with lesional and perilesional psoriasis compared to normal skin (Lee et al., 2012). A similar increase in TEWL was found in patients with acne vulgaris (Yamamoto et al., 1995) and atopic dermatitis (Gupta et al., 2008). A review of several studies by Levin et al. also shows a quantitative relationship with TEWL and percutaneous absorption (Levin and Maibach, 2005).

In vitro percutaneous absorption studies are typically performed on porcine ear skin, rat skin, or human cadaver skin as predictive models during drug development. However, these studies involve the use of healthy skin with intact barrier function. Many topical drug products are intended to be used for the local treatment of skin diseases where the barrier function is compromised. This may result in altered drug delivery into and across the skin and lead to potential systemic toxicity. Thus, *in vitro* studies utilizing compromised skin models may be more relevant in predicting topical drug absorption. Tape stripping is one method which has been used to partially disrupt the stratum corneum to simulate compromised skin. Simonsen et al. observed with TEWL and histological staining that tape stripping 7–25 times partially disrupted the stratum corneum, resulting in increased permeation of betamethasome-17-valerate and fusidic acid across porcine skin (Simonsen and Fullerton, 2007). Delipidization with organic solvents is also a method employed to disrupt the barrier properties of skin by removal of lipids. Tsai et al. observed an increase in TEWL as well as percutaneous absorption of hydrophilic and amphipathic compounds, such as hydrocortisone, caffeine, and sucrose following acetone treatment on hairless mouse skin (Tsai et al., 2001b). Finally, although microneedles are used to enhance transdermal drug delivery (Kim et al., 2012), we utilized them as a method to damage the skin as microneedles essentially function by partially disrupting the stratum corneum.

In addition to the morphology of the skin, the vehicles used in topical formulations also have an influence on the barrier properties of the skin. Specifically, a vehicle may alter the diffusion coefficient of the active or its partitioning into the skin (Cross et al., 2001; Rosado et al., 2003). To our knowledge, no studies have investigated the impact of vehicles on the permeation of

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http://dx.doi.org/10.1016/j.ijpharm.2014.06.050
0378-5173/

compounds through damaged skin. Thus, the objective of our study was to simulate damaged skin with various techniques and determine the influence of vehicle composition on the permeation of a model compound, diclofenac diethylamine, a non-steroidal anti-inflammatory drug used in the treatment of arthritis.

2. Materials and methods

2.1. Chemicals

Diclofenac diethylamine was obtained from Pure Chemistry Scientific Inc. (TX, USA). Light mineral oil, phosphate buffered saline, sodium phosphate monobasic monohydrate, and ortho-phosphoric acid were obtained from Fisher Scientific (MA, USA). HPLC grade methanol was obtained from Medsupply Partners (GA, USA).

2.2. Solubility testing

An excess amount of diclofenac diethylamine was added to 10 mM phosphate buffer saline (PBS) and mineral oil (MO) ($n = 3$ for each). After shaking overnight at room temperature, the samples were filtered using a 0.22 μm syringe filter and diluted accordingly with an organic solvent depending upon the miscibility with the solution. The samples were then analyzed by HPLC.

2.3. Skin preparation

Human dermatomed skin was obtained from a skin bank and stored at −80 °C. Prior to permeation studies, human skin was thawed according to the skin bank instructions. After thawing, the skin was removed from the packets and cut into appropriately sized pieces for permeation. Four groups were tested: intact skin as well as tape stripped, delipidized, and microneedle porated compromised skin. For tape stripping, a transpore tape was placed on top of the skin piece, and a glass rod was rolled over it 20 times. Each tape was then pulled off with equal force using forceps. This process was repeated with 10 tapes to partially compromise the skin barrier. For delipidization, 500 μL of 2:1 chloroform:methanol solution was placed in the donor compartment of mounted skin. The solution remained on skin for 40 min to allow for removal of lipids. The solution was then pipetted off, and the surface of the skin was rinsed with PBS, and any excess was removed with a kimwipe. For microneedle poration, maltose microneedles (3 × 3 array) were pressed into the skin for 1 min to allow for dissolution and formation of the microchannels. All groups studied were $n = 5$.

2.4. Transepidermal water loss measurements

The barrier function of the stratum corneum was determined before and after compromising skin by measuring the trans-epidermal water loss (TEWL) using a VapoMeter (Delfin Technologies Ltd., Kuopio, Finland). The probe was turned on and held on the human cadaver skin for approximately 10 s until a stable reading was recorded. All measurements were carried out in triplicate.

2.5. In vitro permeation studies

Vertical static Franz-type diffusion cells (PermeGear, Hellertown, PA, USA) were used for the permeation studies (Crutcher and Maibach, 1969). The recirculating water bath system was maintained at 37 °C to bring the skin surface temperature to 32 °C. The receptor compartment consisted of phosphate buffer saline at pH 7.4 which could maintain sink conditions. The skin pieces were equilibrated for 15 min. In the donor compartment, 500 μL of 90% saturation solution of diclofenac diethylamine in a given vehicle was added. Skin was dismounted from the Franz cell following 24 h of permeation. Excess donor formulation remaining on the skin was wiped 3 times with Q-tips soaked in PBS, followed by 3 times with dry Q-tips. After 24 h, the skin is hydrated and allows for easy separation of the epidermis and dermis. The epidermis was carefully separated from the dermis with forceps, and each was minced and placed into a scintillation vial with 2 mL of PBS. Samples were placed on a shaker 100 rpm overnight for extraction. A skin extraction study was performed by adding a known amount of drug to the skin and using the extraction method to determine a recovery factor. The extracts were analyzed for drug content by HPLC.

2.6. HPLC assay

HPLC analysis was carried out on Alliance HPLC Waters 2695 Separations Module attached to a Waters UV detector. The HPLC assay was performed using a Phenomenex RP C8 Luna column (250 × 4.6 mm, 5 μ). The mobile phase consisted of 66% methanol and 34% 10 nM sodium phosphate buffer (pH to 3 with *o*-phosphoric acid). The flow rate and injection volume was set to 1.2 mL/min and 10 μL, respectively. The calibration curve was linear ($r^2 = 0.99$) in the concentration range 0.1–100 μg/mL. Diclofenac diethylamine was detected at 276 nm.

2.7. Histological imaging

After compromising skin with methods described previously, the skin was fixed in OCT media (Tissue Tek®) and stored at −80 °C for further analysis. The samples were sectioned into 20 μm sections using a cryostat (Microm HM 550) and placed on a polylysine coated glass slide. The sections were stained with H&E staining and observed under a microscope at 40×. Images were taken using a Nikon Eclipse E600 camera coupled with the microscope.

2.8. Permeability coefficient determination

The steady state flux (J) was determined from the slope of the linear portion of the cumulative amount *versus* time plot. Permeability coefficients were calculated by using the following equation:

$$K_p = \frac{J}{C \times A}$$

where K_p is the permeability coefficient (cm/h), J is the flux at steady state (μg/h), C is the concentration in the donor (μg/mL), and A is the area (cm^2).

2.9. In vivo studies

Male hairless rats 6–8 weeks of age were purchased from Charles River Laboratories. The rats were quarantined for 5 days and observed. The rats were anesthetized with a ketamine/xylazine cocktail (90/10 mg/kg) prior to performing any studies. Tape stripping with 6–8 tape strips (3 M transpore tape) was performed to partially compromise the skin. Aqueous gel (500 mg) or mineral oil (500 mg) with drug at the same level of saturation in each was rubbed onto the entire abdomen. Blood samples were collected at predetermined time points *via* the tail vein. Samples were centrifuged for 10 min at 10,000 rpm to separate the serum.

2.10. LCMS assay

A bioanalytical method was performed according to literature (Lala et al., 2002). Briefly, serum was spiked with ketoprofen as an internal standard. Proteins were precipitated with acetonitrile, and the samples were centrifuged. Hydrochloric acid (0.5 M) was added to the resulting supernatant and vortexed for 5 min.