Pretreatment with Skin Permeability Enhancers: Importance of Duration and Composition on the Delivery of Diclofenac Sodium

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ABSTRACT: The use of chemical penetration enhancers (CPEs) is one of the most common approaches to improve the dermal and transdermal delivery of drugs. However, often, incorporation of CPEs in the formulation poses compatibility and stability challenges. Moreover, incorporation of enhancers in the formulation leads to prolonged exposure to skin increasing the concern of causing skin reactions. This study was undertaken to assess whether pretreatment with CPEs is a rational approach to enhance the permeation of diclofenac sodium. *In vitro* experiments were performed across porcine epidermis pretreated with propylene glycol or oleic acid or their combinations for 0.5, 2, and 4 h, respectively. Pretreatment with combination of oleic acid in propylene glycol was found to enhance the permeation of diclofenac sodium significantly only at 10% and 20% (v/v) level, and only when the pretreatment duration was 0.5 h. Longer durations of pretreatment and higher concentration of oleic acid in propylene glycol did not enhance the permeation of diclofenac sodium. *In vivo* dermatokinetic studies were carried out on Sprague–Dawley rats. A twofold increase in AUC and C_{max} was observed in case of rats pretreated with enhancers over the group that was pretreated with buffer. In conclusion, this study showed that composition of the enhancers and duration of pretreatment are crucial in determining the efficacy of CPEs. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1497–1503, 2014

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INTRODUCTION

The delivery of drugs through skin is an established alternative to other drug delivery systems. The popularity of transdermal drug delivery systems (TDDS) can be attributed to the advantages that it holds over other drug delivery systems. Noninvasiveness, patient compliance, potential for controlled/sustained delivery are few among those many advantages that TDDS has to offer over conventional forms of drug delivery systems. However, skin is less permeable to high molecular weight and polar drugs. Therefore, the number of potential drugs that can be administered transdermally are categorically very small, which emphasizes the need for developing techniques that can improve the permeability of skin. The poor permeability properties of the skin is attributed to the stratum corneum (SC), the "dead" outermost layer of the epidermis.[1,2] Various approaches have been investigated to breach the barrier property of the SC to enhance the permeation of drugs. Generally, these approaches are divided into physical, biochemical, and chemical methods.[3] Iontophoresis, microneedles, prodrugs, and barrier perturbation with chemical penetration enhancers (CPEs) are the techniques that are employed either singly or in combination to improve drug delivery across the epidermis.[4]

Stratum corneum is an arrangement of corneocytes embedded in a lipid cast. This pattern gives SC the property to be confined to the external environment. CPEs have the ability to reversibly modulate the SC barrier and thereby improve uptake of permeants.[5] Earlier studies on the mechanism of the action of CPEs has suggested that most enhancers act primarily on the lipidic regions of the SC, thereby promoting easy permeation of drug molecules. Additionally, protein components in the corneocytes contribute to the overall barrier property of the SC. Some enhancers are also known to interact with the protein components, thus assisting better permeation. The enhancers that can simultaneously act on the lipid and the protein regions is likely to be more effective.[5,6] Generally, CPEs are incorporated along with the transdermal formulation.[7] However, incorporation of CPEs often poses formulation problems such as immiscibility, incompatibility, and interactions. The other alternative method to promote the percutaneous absorption of drugs is to pretreat the skin with those CPEs that perturb the SC barrier.

The primary purpose of the present paper is to rationalize that pretreatment of epidermis is a potential and pragmatic approach to enhance permeation of drugs across the epidermis. The effect of pretreating the skin with different concentrations of oleic acid in propylene glycol on percutaneous absorption of diclofenac sodium, a widely used nonsteroidal anti-inflammatory drug, was investigated in this project. Permeation studies were carried out with aqueous solution of diclofenac sodium; however, to substantiate the pretreatment approach for achieving enhancement, permeation was also performed with HPMC (hydroxypropyl methylcellulose) gel system incorporated with 1% diclofenac sodium and a commercial diclofenac sodium Voltaren® gel formulation. Further, to assess the feasibility of this approach, dermatokinetic profile of the drug was evaluated by cutaneous microdialysis in rats.

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MATERIALS AND METHODS

Diclofenac sodium, oleic acid, isopropyl myristate, propylene glycol, 1-phenyl piperazine, and phosphate-buffered saline (PBS; pH 7.4) were purchased from Sigma–Aldrich Inc. (St. Louis, Missouri). Ag/AgCl wire was purchased from Alfa Aesar (Ward Hill, Massachusetts). All other chemicals and reagents used were of analytical grade. All solutions were prepared in deionized water.

Diclofenac sodium gel (1%) was prepared by dissolving HPMC (Methocel E4M premium) powder in one-fifth of the required total amount of water as hot water with continuous agitation until a uniform dispersion is obtained. Drug was dissolved in remainder of the water and was added to the polymer solution with continuous stirring. The mixture was left overnight for effecting complete hydration of the polymer.

Epidermis Preparation

Porcine whole skin from the abdominal region was obtained from a local abattoir. The hair from the skin was shaved off using an electric razor and all the adhering subcutaneous fat and exogenous tissues were removed carefully. Skin was cut into small pieces and wrapped into aluminum foil and then was immersed in water maintained at 60°C for 2 min following which the epidermis was carefully teased off from the dermis. The peeled epidermis was mounted onto glass slides and stored at 4°C. The stored epidermis was used within 3 days.

Experimental Setup for *In Vitro* Permeation Studies

In vitro permeation studies were performed across porcine epidermis with Franz diffusion cells. Prior to use, the epidermis was thawed at room temperature for 1 h. The epidermal membrane was carefully mounted on the Franz diffusion cell (SC facing the donor side) having a receiver volume capacity of 5 mL and was fastened with a rigid clamp. The donor and receiver compartment was filled with PBS (pH 7.4). The integrity of epidermis was checked before starting the experiment by measuring the resistance at a frequency of 10 Hz and low voltage of 100 mV. The epidermis having resistance value greater than 20 KΩ/cm^2 only was used for permeation studies. During permeation studies, the receiver compartment buffer was stirred throughout the experiment to maintain sink conditions. This setup was maintained at 37°C by a water circulator.

Pretreatment of Epidermis

After measuring the initial electrical resistance, the donor compartment was replaced with 0.5 mL of enhancer solution following which the donor chamber was sealed off with a parafilm. The CPEs used for pretreatment were oleic acid or propylene glycol or their combinations. The enhancer solution was kept in contact with the epidermis for 0.5, 2, and 4 h. Following pretreatment, the enhancer solution was discarded and the epidermis carefully washed with methanol and wiped off with cotton swabs to remove any adhering enhancer solution. Permeation studies were carried out across the epidermis that was pretreated for 0.5, 2, and 4 h, respectively, with oleic acid or propylene glycol in their neat form or their combinations. Epidermis pretreated with PBS for 0.5, 2, and 4 h was used as a control.

In Vitro Permeation Studies

Permeation was carried out by placing a saturated solution of diclofenac sodium or 1% diclofenac sodium HPMC gel or a 1% Voltaren® gel formulation in the donor chamber for 24 h, and samples were withdrawn at different time points from the receiver compartment and analyzed using HPLC.[8]

Extraction of Diclofenac Sodium from Epidermis

At the end of permeation studies, any adhering formulation to the epidermis was removed by washing it with methanol and water. The active diffusion area (0.64 cm^2) of the epidermis was cut off using a biopsy punch and weighed. The epidermis was then homogenized in methanol using a tissue homogenizer (Tissuemiser; Fischer Scientific, Pittsburgh, PA). This solution was kept on a Labquake™ shaker for 24 h for effecting complete extraction of diclofenac sodium. Thereafter, the solution was centrifuged for 15 min at 1027 g. The supernatant was collected and was directly injected into HPLC to measure the content of diclofenac sodium in the epidermis. The validity of this procedure was established by spiking known amounts of diclofenac sodium in blank homogenates of epidermis–methanol solution followed by an extraction procedure similar to above. The percentage recovery was found to be greater than 98%.

Dermal Microdialysis in Rats

Microdialysis performed in Sprague–Dawley rats (200–250 g) was an adaptation of a previously reported procedure.[9] The animal studies were approved by the Institutional Animal Care and Use Committee (IUCAC) at the University of Mississippi (Protocol #11-016). The hairs from the abdominal region of the skin were shaved off with clippers 1 day prior to the study. On the day of experiment, rats were anaesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) by an intraperitoneal injection. The dorsal region of the skin was punctured with a 20-gauge needle. A linear microdialysis probe having a 5-mm membrane length with a molecular weight cut off of 30 kDa was inserted through the needle. Thereafter, the needle was removed leaving the dialysis window implanted in the dermal region. The probe was then equilibrated with isotonic PBS (perfusate pH 7.4) for 1 h. During the entire length of the study, PBS (perfusate pH 7.4) was continuously perfused through the probes at a flow rate of 2 μL/min. A cylindrical chamber with an area of 1.77 cm^2 was glued to the rat skin at the site of probe implantation, and pretreatment of the skin was effected by placing the enhancer solution in this chamber. After 0.5 h, the enhancer solution was removed from the surface, wiped off with cotton swabs and washed with methanol. This was followed by application of 1% diclofenac sodium HPMC gel (1 g) at the pretreated skin area and performing microdialysis for 8 h. At this point, the gel was removed from the application site and microdialysis was further continued for another 4 h. A similar procedure was followed when gel was applied topically to the skin pretreated with PBS (control) for 0.5 h and the drug sampled by microdialysis. The probe recovery was determined *in vivo* by retro-dialysis method where a known drug concentration solution in PBS was perfused through the probe and dialysate collected every hour for 2 h and analyzed by HPLC. The loss of the drug from the perfusate to the extracellular fluid represents the percentage recovery that is calculated using the recovery formula.[9,10]

$$(\%)\,\text{Recovery} = 100 - \left(\frac{\text{Concentration of dialysate}}{\text{Concentration of perfusate}} \times 100\right)$$