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PERMEATION STUDY

Evaluation of the Percutaneous Absorption of Ketoprofen, Diclofenac Sodium, and Lidocaine Hydrochloride in VersaPro™ Cream and Gel

INTRODUCTION

Chronic pain is one of the most common reasons adults seek medical attention and affects between 15-30% of the general adult population. Roughly 20.4% (50 million) of American adults had chronic pain in 2016 according to the National Health Interview Survey (NHIS) and 8% of U.S. adults (19.6 million) had high-impact chronic pain, with higher prevalence of both chronic pain and high-impact chronic pain. This health condition has been linked to restrictions in mobility and daily activities, dependence on opioids, anxiety and depression, poor perceived health and reduced quality of life. Furthermore, chronic pain has contributed an estimated $600 billion each year in direct medical costs, lost productivity and disability programs in the US. [1,2,3]

There are several pharmaceutical options for the treatment of chronic pain, available as oral or topical therapies. The two major classes of oral analgesics used for treating pain are opioids and nonsteroidal anti-inflammatory drugs (NSAIDs). The use of local anesthetics have many potential advantages in situations where the cause and source of the pain is limited to a particular site or region. [4] Because side effects such as gastric disturbances, cardiovascular complications, renal dysfunction, first-pass hepatic metabolism and variable serum concentrations are associated with the oral use of analgesics, an alternative local delivery of drugs by applying transdermal dosage forms including gels and creams could provide several benefits to patients treating chronic pain. [5] Furthermore, some clinical trials suggest that in the head-to-head comparison of topical and oral NSAIDs, topical NSAIDs are as effective on patients with less side effects as compared with those using oral formulations. [6,7,8]

Transdermal drug delivery is a non-invasive method of delivering drugs systemically by applying a drug formulation onto intact and healthy skin. The drug initially penetrates through the stratum corneum and then passes through the deeper epidermis and dermis without drug accumulation in the dermal layer. When the drug reaches the dermal layer, it becomes available for systemic absorption via the dermal microcirculation. [9] Several studies have demonstrated that the use of transdermal formulations containing NSAIDs such as Diclofenac sodium and Ketoprofen combined with anesthetics such as Lidocaine HCl leads to high drug concentration at the site of application while maintaining low systemic adverse effects and no drug-drug interactions. [10, 11, 12] Moreover, the combination of multiple drugs such as analgesics and anesthetics in transdermal preparations may lead to a synergistic effect and potentially enhance pain relief, consequently resulting in better patient compliance. [13]

The present study aimed to evaluate the skin permeation and penetration of two multiple drug formulations containing Ketoprofen 10% w/w, Diclofenac sodium 3% w/w and Lidocaine HCl 2% w/w using a new generation of transdermal bases -VersaPro™ Gel and VersaPro™ Cream- through a human skin *in-vitro* percutaneous absorption experiment.

MATERIALS AND METHODS

Chemical Reagents

Diclofenac sodium USP, Ketoprofen USP, Lidocaine HCl USP, Ethoxy Diglycol, Pentylene Glycol, VersaPro™ Gel Base and VersaPro™ Cream Base were provided by Medisca Pharmaceutique Inc. (St. Laurent, QC, Canada). High performance liquid chromatography (HPLC) grade solvents were used for sample analysis and provided by Rutgers University (New Jersey, NJ, US).

Preparation of Drug-Loaded Formulations

The samples consisted of Ketoprofen 10% w/w, Diclofenac sodium 3% w/w and Lidocaine HCl 2% w/w in VersaPro™ Gel and VersaPro™ Cream bases. These formulations were prepared using a planetary mixer MAZ® (Mazerustar KK-400, Kurabo, Japan). Firstly, Lidocaine

HCl was added to the stainless steel MAZ® jar and milled at 1000 rpm for 60 seconds (2 x 30 sec) using approximately 50 Zirconia beads. After milling, the micronized Lidocaine HCl, Diclofenac sodium and Ketoprofen were levigated with Ethoxy Diglycol/Pentylene Glycol (50/50) at 2000 rpm for 2 minutes. At the end, either VersaPro™Gel or VersaPro™ Cream was added and mixed at 2000 rpm for 2 minutes. The resulting preparations were then evenly separated into top, middle and bottom samples, and tested to ensure integrity of drug products following USP Chapter <3>.

Quantification of Drugs by LC/MS

A high-performed liquid chromatography/mass spectrometry method was previously developed and validated for Ketoprofen, Diclofenac sodium, and Lidocaine HCl content determination. A Dionex Ultimate 3000 RLSC nanosystem (ThermoFisher, Sunnyvale, CA) was used in the LC/MS system. Isocratic chromatographic separation was performed on CMP scientific C18 column (50 x 2.1mm, 3.5µm), using the mobile phase of acetonitrile-water-formic acid (60:40:0.1, v/v/v) which was delivered at a flow rate of 0.2 mL/min. The column temperature was maintained at room temperature (25℃). The sample injection volume was 5 µL. A LTQ Orbitrap Velos mass spectrometer (ThermoFisher, San Jose, CA), equipped with an electrospray ionization (ESI) source, was used in positive ion mode with full MS for the quantitative analysis. The spray voltage was set to 4.0 kV, and the capillary temperature was maintained at 274℃. Quantification was performed using the transitions *m/z* 255.2→209.1 for Ketoprofen, *m/z* 296.1→278.0 for Diclofenac sodium, and *m/z* 335.3→86.2 for Lidocaine HCl. Limit of quantification was set at 1 mg/mL.

***In-vitro* permeation study**

For each formulation, an *in-vitro* test using Franz diffusion cells was carried out for the purpose of evaluating the permeation of Ketoprofen, Diclofenac sodium and Lidocaine HCl from VersaPro™ Gel and VersaPro™ Cream formulations into the skin. Phosphate Buffer (PBS), pH 7.4 was used as the receptor solution and human abdomen skin tissue collected from living female donors (thickness 750µm) was used as the biological membrane. The skin was defrosted by soaking in PBS, pH 7.4 at room temperature for 15-30 minutes and then cut into pieces of approximately 2.5 cm^2 using sterilized scissors. Clean Franz Diffusion cells were assembled and labelled and the skin was sandwiched between the donor and receptor chambers. The receptor compartment was filled with 5 mL of PBS, pH 7.4 and a magnetic stir bar was added to the chamber. The cells were maintained at 32℃ ± 0.5 in a thermostatic bath at 600 rpm. Each cell was checked for bubbles under the skin and skin integrity was measured in terms of transepidermal water loss (TWEL) using a Vapomer (Delfin Technologies). Only skin pieces of TWEL below 20 $g/m^2/h$ were selected for the permeation study. A finite dose of 5 mg/cm^2 was applied on the donor membrane by using a glass rod and a diffusion area of each donor compartment corresponded to approximately 2.0 cm^2.

After applying samples, an aliquot of 300 µL of receptor solution was sampled at four time points: 4h, 9h, 19h and 24 h for HPLC/MS analysis of each drug content. The same volume was replaced with fresh buffer to maintain sink conditions. To calculate the cumulative amount of Ketoprofen, Diclofenac sodium and Lidocaine HCl permeated (Q) through the skin at time t, dilutions obtained after the first collection were considered, and the following equation was used according to Sato *et al*. (2007) [14].

$$Q = C_{measured} \times V_r + \sum^{n-1} \times C_a \times V_a \quad (1)$$

Where $C_{measured}$ is the concentration of the sample at time t, V_r is the volume of receptor solution of the diffusion cell, C_a is the concentration of the sample removed, V_a is volume of the sample removed. Cumulative permeation curves expressed in ng/cm^{-2} of permeated drug versus time (hour) were then generated for achievement of the permeation profiles. The steady-state permeation flux (*Js* [$ng\ cm^{-2}\ h^{-1}$]) was determined from the slope of the linear portion of the cumulative amount of each drug versus the time curve. The permeability coefficient (*Kp*, $cm\ h^{-1}$) was calculated from the drug flux divided by the applied drug concentration in the donor compartment.

Cutaneous Distribution in the epidermis and dermis

After 24 hours of permeation testing, the system was dismantled, and the skin was removed from the diffusion cells and rinsed twice with 5 mL of PBS, pH 7.4. The portion of the skin exposed to the formulations was cut as a circular piece and the epidermis was separated from the dermis using tweezers. Both epidermis and dermis were subjected to the extraction of the drugs by adding 1 mL of methanol at 3000 rpm and homogenized for 3 cycles of 180 seconds (Bead Bug Homogenizer, Benchmark Scientific). Samples were then centrifuged at 10000 rpm for 5 min. Supernatant was filtered using a 0.45 µm syringe filter and samples were analyzed by LC/MS.

As a result, a graph representing dermal, epidermal and reception media distribution of Ketoprofen, Diclofenac and Lidocaine HCl was generated. Results were used to evaluate the ability of VersaPro™ Gel and VersaPro™ Cream as penetration enhancers of these drugs through different skin structures.

Statistical Analysis

All the experiments were carried out in five replicates (n = 5). The results of percutaneous absorption were presented as cumulative amount (mean ± standard deviation) of Ketoprofen, Diclofenac sodium and Lidocaine HCl that was measured in the receptor solution. Differences in percutaneous absorption and dermal/epidermal distribution between our two compounded formulations were evaluated using analysis of variance (ANOVA). Statistical differences were considered significant when p values < 0.05 with a Tukey-Kramer post-hoc test to compare each time point.

RESULTS AND DISCUSSION

As the knowledge of pain pathways has increased, so has the tailoring of drugs and alternative dosage forms to act on these mechanisms making them safer and more effective. After penetration and permeation of the skin, the drugs are able to directly act on the initiating factors that lead to pain. Most advantageously, the low incidence of adverse reactions after the topical administration of a drug substance

is a particularly important factor in the therapy of chronic conditions. This can be of great benefit for those patients requiring pain medication for a prolonged period of time. [15,16, 17]

The *in-vitro* Franz human skin dose model has proven to be a valuable tool for the study of percutaneous absorption and the determination of the cutaneous distribution of topically applied drugs. The model uses *in-vitro* human skin, most often trunk skin, mounted in specially designed static diffusion chambers allowing the skin to be maintained at a temperature and humidity that match typical *in vivo* conditions. [18]

Based on the known advantages of applying pain medication topically, this study was designed to evaluate the percutaneous absorption profiles and cutaneous distribution of two compounded formulations containing 10% Ketoprofen, 3% Diclofenac sodium and 2% Lidocaine HCl mixture using VersaPro™ Gel and VersaPro™ Cream bases across human abdominal skin in a Franz diffusion cell.

Before samples were tested for permeation and penetration, it was observed that the two formulations were compounded successfully using the MAZ® planetary mixer. Both gel and cream preparations were homogeneous and generated a smooth feel when rubbed onto the skin surface, hereby ensuring the integrity of the product before permeation testing.

Permeation curves over time were built to evaluate the permeation profiles of Ketoprofen, Diclofenac sodium and Lidocaine HCl based on the cumulative amount of drug at pre-established time points 4, 9, 19 and 24h (Figures 1, 2 and 3). According to the results obtained in this study, all drug permeation profiles were characterized by an expressive rise between 4 hours and 24 hours following topical application in both dosage forms. Furthermore, Ketoprofen, Diclofenac sodium and Lidocaine HCl compounded in VersaPro™ Gel were shown to deliver the active substances across the skin barrier with a steady-state permeation flux of 2395.47, 224.16, and 353.7 ng/cm^2/h respectively. The steady-state flux for the same drugs compounded in VersaPro™ Cream were 2838.28, 315.36, and 490.33 ng/cm2/h respectively. No significant differences in permeation profiles were observed between the cream and gel formulations for each drug tested.These results

Ketoprofen Permeation Profiles

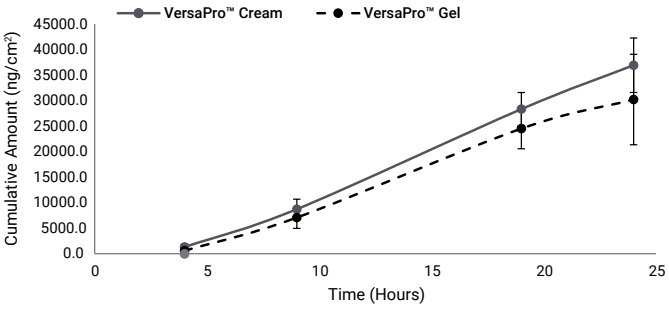

FIGURE 1 – Cumulative amount of Ketoprofen from VersaPro™ Gel and VersaPro™ Cream formulations over time. The above represents the average cumulative amount of Ketoprofen (ng/cm^2, Mean ± SD) transferred by a diffusion area using human skin as the membrane model monitored for 24 hours (n = 5).

Diclofenac Sodium Permeation Profiles

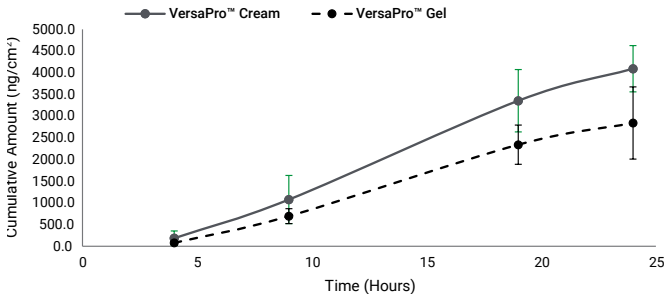

FIGURE 2 – Cumulative amount of Diclofenac sodium from VersaPro™ Gel and VersaPro™ Cream formulations over time. The above represents the average cumulative amount of Diclofenac sodium (ng/cm^2, Mean ± SD) transferred by a diffusion area using human skin as the membrane model monitored for 24 hours (n = 5).

Lidocaine HCl Permeation Profiles

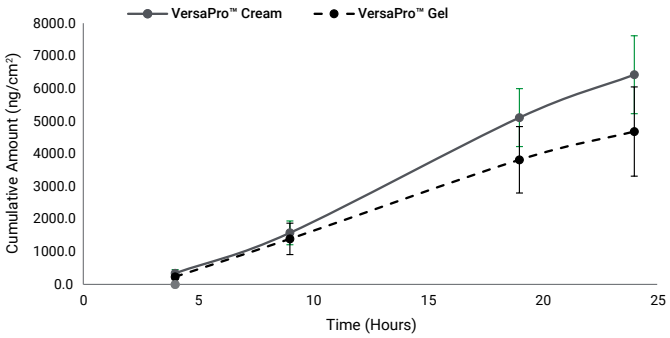

FIGURE 3 – Cumulative amount of Lidocaine HCl from VersaPro™ Gel and VersaPro™ Cream formulations over time. The above represents the average cumulative amount of Lidocaine HCl (ng/cm^2, Mean ± SD) transferred by a diffusion area using human skin as the membrane model monitored for 24 hours (n = 5).

clearly indicate that VersaPro™ Gel and VersaPro™ Cream are both effective delivery vehicles for multiple active substances evaluated through human abdominal skin over time.

The success of topical drug delivery depends on its ability to effectively overcome biological barriers. According to Jankowski et al [19], hydrogels and emulsions may be used as permeation enhancer vehicles for different drugs. The skin hydration and incorporation of some semi-solid base components such as surfactants, emollient, liquid crystal ingredients and moisturizing agents into the intercellular cement lipids would lead the increased disordering of lamellar and lateral packing of lipids and/or increased solubility of the active substance within the stratum corneum lipids. These interactions may alter the stratum corneum permeability (influence on skin penetration and permeation rate) or change the value of skin/base partition coefficient (influence on the rate of the skin retention). These factors would be the most likely cause for the delivery of the drugs into the receptor compartment using VersaPro™ Gel and VersaPro™ Cream bases in this study.

Following the permeation experiments, the amount of each drug was quantified in different skin layers including the receptor compartment after their extraction from the human skin. The skin distributions of Ketoprofen, Diclofenac sodium and Lidocaine HCL in epidermis, dermis and receptor medium are displayed in figures 4 and 5.

Skin Distribution of Ketoprofen, Diclofenac Sodium and Lidocaine HCl in VersaPro™ Cream

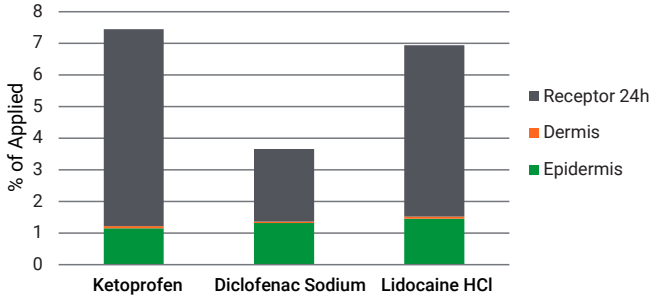

FIGURE 4 – Skin distribution of Ketoprofen, Diclofenac sodium and Lidocaine HCl in epidermis, dermis and receptor media after 24h application of a formulation containing Ketoprofen 10%, Diclofenac sodium 3% and Lidocaine HCl 2% in VersaPro™ Cream. (n=5).

Skin Distribution of Ketoprofen, Diclofenac Sodium and Lidocaine HCl in VersaPro™ Gel

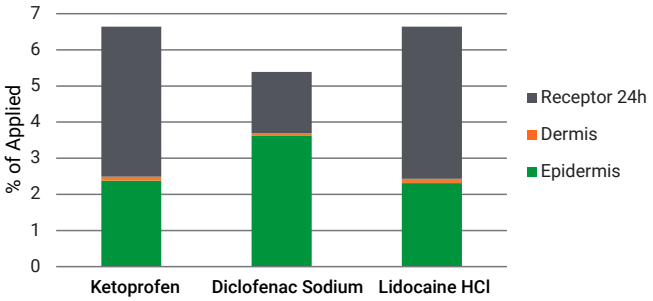

FIGURE 5 – Skin distribution of Ketoprofen, Diclofenac sodium and Lidocaine HCl in epidermis, dermis and receptor media after 24h application of a formulation containing Ketoprofen 10%, Diclofenac sodium 3% and Lidocaine HCl 2% in VersaPro™ Gel. (n=5).

Overall amount of drug that penetrated through the skin should be measured not only by the drug quantity present in the receptor compartment, but also by the quantity of drugs retained in different skin layers such as the dermis and epidermis. Once the drug reaches the dermis, a vascularized skin layer, it will be able to reach the bloodstream and then distributed systemically. [20] In our study, the three drugs were found in the skin layers and in the receptor medium. This revealed that after the application of the samples on the skin, each drug crossed the stratum corneum following its path of passive diffusion through the skin structure until the receptor medium.

CONCLUSION

To date, studies in regard to transdermal absorption of multiple drugs have been limited. This study evaluated the permeation and percutaneous absorption of Ketoprofen, Diclofenac sodium and Lidocaine HCl mixture in VersaPro™ Gel and VersaPro™ Cream. The permeability results showed an increased permeation profile of the drugs over time and the ability of these three drugs to permeate through abdominal human skin *in-vitro* when compounded into these two pre-made transdermal bases. The skin distribution of the drugs also showed that all actives were able to overcome the stratum corneum and reach the skin layers and receptor medium. The results obtained from this study clearly indicate that VersaPro™ Gel and VersaPro™ Cream can successfully deliver local and systemic levels of the evaluated drugs following topical application. In addition, the formulations contained multiple drugs in a single dosage form, hereby these results might help to promote a better patient compliance using a single compounded medication.

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