The Effect of Formulation Excipients on the Penetration and Lateral Diffusion of Ibuprofen on and within the Stratum Corneum Following Topical Application to Humans

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Received 30 September 2013; revised 16 December 2013; accepted 16 December 2013

Published online 13 January 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23850

ABSTRACT: Distribution profiles of topically applied drugs can be influenced by the presence of excipients. This study investigated the effect of common topical excipients on the simultaneous lateral diffusion and stratum corneum (SC) penetration of a model compound, ibuprofen (IBU) in humans. IBU solutions with and without propylene glycol (PG), polyethylene glycol 200 (PEG 200), and/or octisalate (OS) were dosed onto the forearm of participants. At various times, 10 "tape-strippings" were obtained with perforated concentric tapes and analyzed for IBU concentration and SC protein mass. Complimentary *in vitro* permeation studies assessed the effect of excipients on the percutaneous absorption of IBU across human skin. Following *in vivo* application, IBU displayed a greater tendency for lateral diffusion when applied with OS, whereas IBU resisted lateral diffusion when dosed with PG and PEG 200. After 24 h, 25.3 ± 8.0% and 55.5 ± 18.6% of IBU was recovered from the SC *in vivo* with and without excipients, respectively. There was a twofold–to threefold enhancement in IBU flux *in vitro* when applied with excipients. The lower IBU recovery from the SC when applied with excipients may be attributed to the permeation enhancement effects of these excipients. The ability of IBU to laterally diffuse appears to be dependent on formulation excipients. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:909–919, 2014

Keywords: penetration; lateral diffusion; tape stripping; percutaneous absorption; ibuprofen; transdermal drug delivery; transdermal; diffusion; permeability; absorption enhancer

INTRODUCTION

Transdermal delivery has proven to be an effective approach to deliver drugs for the purpose of achieving a local or systemic effect. However, penetration of drugs through the skin is often limited by the barrier properties of the stratum corneum (SC). Although it is accepted that the bioavailability of drugs delivered through the skin is generally less than 10%,[1] the need to establish the fate of the unabsorbed drug is often ignored. The portion of unabsorbed topically applied drug is assumed to be shed via epidermal desquamation, metabolized, or washed or rubbed from the skin. However, very few studies have recognized that in addition to drug penetration through the skin, the process of lateral drug diffusion across the skin[2–6] may be an additional source of reduced drug recovery.

Following drug application onto the skin, an applied drug can diffuse laterally on the surface of the SC, along the "horizontal" plane of the lipid bilayers within the SC, as well as being transported from one bilayer into a parallel bilayer through adjacent lipid channels between the corneocytes.[6] Lateral diffusion may therefore increase the area of skin exposure to the drug, which is reported in literature to be of crucial importance when assessing the distribution behavior of topically applied substances.[3,7,8] From a safety point of view, lateral diffusion also has the potential to increase the risk of secondary drug transfer to third parties by migrating beyond the site of application. To investigate this phenomenon, we previously developed a method to better understand the factors affecting the simultaneous processes of lateral drug diffusion and drug penetration across the skin*in vivo*. [6] Using this novel approach, we identified that the physicochemical properties of topically applied drugs appear to dictate the degree of their lateral diffusion across the skin. However, as most drugs are often applied to the skin in conjunction with various formulation excipients, it is also necessary to determine the impact of such excipients on the lateral diffusion of drugs to better understand the fate of topically applied drugs from real formulations. Common excipients that are used in topical formulations, and were therefore considered in this investigation, include propylene glycol (PG), polyethylene glycol 200 (PEG 200), and the penetration enhancer and sunscreen, octisalate (OS).

Penetration enhancers are chemicals that are able to reversibly reduce the barrier function of the SC without damaging viable cells. It is generally thought that they exert their effect by extracting lipids from the skin, thereby creating diffusion pathways through which drugs may permeate, or by intercalating between lipid bilayers to create spatial disruption and lipid fluidization.[9–11] The sunscreen agent OS has been demonstrated by Morgan et al.[12] to enhance the skin permeation of hormones across the skin. It has been suggested that the possible lipid fluidizing effect of OS increases the free volume within the SC lipid bilayers thereby facilitating partitioning of permeants into the SC.[10]

Propylene glycol has also been shown to improve the skin permeation of compounds when used alone[13] or when used as

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Journal of Pharmaceutical Sciences, Vol. 103, 909–919 (2014)

a cosolvent to produce a synergistic effect.[14–16] The mechanism by which such cosolvent systems increase transdermal flux has been attributed to increasing the thermodynamic activity of the permeant within the SC, interacting with the SC to increase drug solubility within the SC, or reducing the resistance of the transport pathway.[17,18]

Polyethylene glycols are widely used as emollients and solvents in cosmetic formulations. In pharmaceutical products, their function can be extended to that of a penetration enhancer. By decreasing the surface tension of the SC, PEGs may enable or enhance the diffusion of other molecules through the skin.[19] However, a reduction in skin permeability has also been demonstrated following application of PEGs by the formation of complexes and/or their ability to increase the viscosity[20] of formulations.

Although there have been many studies assessing the individual effects of the above-mentioned excipients on penetration, the potential synergistic effects of OS, PG, and PEGs on the penetration enhancement and lateral diffusion of model compounds across human skin *in vivo* and *in vitro* have yet to be explored. Importantly, an understanding of the impact of such excipients on the fate of the drug is crucial as it may provide insight into more appropriate methods to increase penetration and reduce drug loss (and subsequent secondary drug exposure). Therefore, the aim of this study was to determine whether different combinations of the hydrophilic PG and PEG 200 and the lipophilic OS influenced the *in vivo* lateral diffusion and penetration behavior of a model nonsteroidal anti-inflammatory drug ibuprofen (IBU) across human skin. This was assessed using a novel adhesive tape design previously developed in our laboratory whereby the tape was perforated into concentric rings.[6] *in vitro* permeation studies across excised human skin were also undertaken to assist in the interpretation of the data obtained by tape stripping data from *in vivo* human studies.

MATERIALS AND METHODS

Materials

Ibuprofen, OS, PG, bovine serum albumin (BSA), and Bradford reagent were obtained from Sigma–Aldrich (Castle Hill, New South Wales, Australia). Ethanol (90%, v/v) (EtOH), methanol (MeOH), sodium hydroxide, hydrochloric acid (36%, v/v) (HCl), PEG 200, and phosphoric acid were purchased from Merck (Kilsyth, Victoria, Australia). Potassium dihydrogen orthophosphate was obtained from Univar (Ingleburn, New South Wales, Australia) and purified water was purchased from a Milli-Q™ water purification system (Millipore, Bedford, Massachusetts).

Formulations

Propylene glycol, PEG 200, and OS were added to ethanolic solutions of IBU (5%, w/v) to assess their effect on the penetration and lateral diffusion of IBU at various exposure times. Hence, the formulations applied in this study consisted of ethanolic solutions of the following components: IBU, IBU + PG, IBU + OS, and IBU + PG + PEG 200 + OS. These combinations were of particular interest to our industry partner—Acrux Ltd. (Melbourne, Australia) and were thus selected to investigate their potential effects on the penetration enhancement and lateral diffusion within the SC (as well as lateral spreading on the surface of the SC) of IBU across human skin *in vivo* and *in vitro*.

Octisalate was incorporated into the formulations at 5% (w/v) to match the concentration that is commonly used in sunscreens, whereas PG and PEG 200 were present in formulations at a concentration of 10% (w/v), as it has been suggested that concentrations above 10% (w/v) are capable of producing skin sensitization.[21–23]

Human Subjects

For studies conducted over 6 h, eight participants (four males and four females) provided consent to take part in the study approved by the Standing Committee on Ethics in Research Involving Humans (SCERH), Monash University, Victoria, Australia (Project #CF08/1125–2008000555). Studies conducted over 24 h were performed on three healthy volunteers (one male and two female) who had also provided consent to take part in the research. The volunteers consisted of a combination of Asians, Africans, and Caucasians, who had no history of skin disease and were aged 24–29 years. All volunteers were requested to avoid applying any topical products to their left and right flexor forearms for at least 48 h before the commencement of the study.

Topical Application

The left and right flexor forearm of each volunteer was decontaminated and prepared for the study as outlined by Gee et al.[6] A finite dose of ethanolic solution containing IBU with and without PG, PEG 200, and/or OS was dosed onto individual premarked application sites of the flexor forearm using a HPLC manual injection syringe to deliver 1.8 μL and allowed to air dry while the forearm rested on a bench. It should be noted that each formulation was applied on separate days, at least 1 week apart.

Tape Striping Collection Time Points

Visual inspection confirmed that the small dose of EtOH evaporated soon after application (approximately 10 s). Therefore, application sites were tape stripped at 3 min, 3 h, or 6 h after application. Sites dosed with IBU with and without PG, PEG 200, and OS were also tape stripped at 24 h after application. One application site was assigned for each formulation per time point for each participant. The participants were requested to leave the application areas unoccluded between sampling time points and to wear short-sleeved clothing so as to minimize the potential for loss of drug onto clothing. For those studies exceeding 6 h in duration, a metal tea leaf strainer made of wire mesh and with a cross-sectional area of 10 cm^2 was placed over the sample site (following the 6 h tape strip) and secured onto the skin with Tegaderm® (3M, St Paul, Minnesota) as shown in Figure 1. The strainer was applied to prevent volunteers from rubbing any drug still adhered to the SC during the 6–24 h exposure time.

Over the course of the sampling time points, volunteers were also requested not to participate in activities that would cause perspiration as this may affect the diffusion and penetration behavior of the IBU applied. In addition, participants of the 24 h study agreed not to bath or shower for 24 h after the formulations were dosed onto their forearm to prevent any IBU from being washed off. The strainer was removed from the volar forearm before tape stripping at 24 h.

 DOI 10.1002/jps.23850