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Investigation into the Mechanism by Which Cyclodextrins Influence Transdermal Drug Delivery

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The objective of this study was to investigate the mechanism by which hydroxypropyl-β-cyclodextrin (HPCD) increases transdermal permeation. Hairless mouse skin was pretreated with HPCD solutions for up to 4 h. After removing the HPCD, corticosteroid-containing suspensions were applied and the transdermal flux and skin accumulation of two model drugs were investigated. After pretreatment, changes to the stratum corneum endothermic melting transitions were determined as an indication of HPCD-induced lipid disorganization. Results demonstrated that HPCD pretreatment had no significant effect on the transdermal permeation or skin accumulation of the model corticosteroids. These findings suggest that HPCD functions to enhance the apparent solubility of the drug in the formulation, thus increasing transdermal permeation rather than extracting lipids from the skin.

Keywords hydroxypropyl-β-cyclodextrin; corticosteroids; transdermal permeation; skin accumulation; epidermal lipids disruption

INTRODUCTION

Because of its accessibility, skin has long been considered a route for systemic drug administration, and research into transdermal drug delivery has expanded greatly over the last several decades. The major obstacle to systemic drug absorption through the skin is the stratum corneum (SC), the outermost layer of the skin, which is composed of keratin-filled corneocytes embedded in a complex mixture of lipids. The major components of the lipids are ceramides, cholesterols, and fatty acids (Elias, 2005). There are two routes that a drug can take to penetrate across the SC: intercellular or transcellular. Nonpolar drugs preferentially penetrate through the continuous intercellular lipid phase of the SC. In contrast, polar drugs predominantly use the hydrophilic pathway, passing through the cells and intercellular proteins to transverse the SC (Sznitowska, Janicki, & Williams, 1998; Weigmann et al., 2005). Because most drug substances are relatively lipophilic, the intercellular lipid components are important determinants in the penetration process.

Generally, the rate at which a lipophilic drug moves across the SC is dependent on two factors: (1) their ability to dissolve in the formulation vehicles, which determines the concentration presented to the absorption site; and (2) their ability to permeate the SC barrier, which is governed by the octanol–water partition coefficient ($P_{o/w}$). Drugs with molecular weights ranging from 100 to 800 and of moderate lipophility (log $P_{o/w}$ = 2) are expected to exhibit a high transdermal penetration (Benson, 2000). The daily dose of drugs that can realistically be delivered from a transdermal patch is 5–10 mg, which is not sufficient for a number of potential therapeutic agents (Benson, 2005). Thus, various methods have been developed to improve transdermal permeation: that is, increasing the thermodynamic activity of the drug in the formulations, or altering physicochemical properties of the SC (Brown, Martin, Jones, & Akomeah, 2006).

Cyclodextrins have recently been employed to enhance transdermal drug delivery. These compounds are crystalline, cyclic oligosaccharides with a bucket-like structure, having a hydrophobic internal cavity and a hydrophilic exterior. This unique shape allows for the formation of inclusion complexes, where a second, lipophilic compound is noncovalently bound within the cavity. Complexation between cyclodextrins and a drug substance is based on hydrogen bonding or van der Waals interactions, and therefore the drug may be easily dissociated. Hydroxypropyl-β-cyclodextrin (HPCD) is a chemically modified cyclodextrin and is one of the most commonly used cyclodextrins in pharmaceutical applications due to its high inclusion capacity and aqueous solubility (Rowe, Sheskey, & Owen, 2006). There are many examples of transdermal formulations in which the active drug substances are complexed within the HPCD cavity as a method to enhance drug transdermal absorption (Loftsson & Brewster, 1996; Murthy, Zhao, Sen, & Hui, 2004). Because of the large molecular size of HPCD, this species and its inclusion complexes with the active drug do not readily penetrate into the skin. Therefore, HPCD does not enhance transdermal penetration by a carrier mechanism within the SC. The majority of published papers support the hypothesis that HPCD increases transdermal permeation through increased

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aqueous solubility, creating a greater thermodynamic driving force for penetration (Felton, Wiley, & Godwin, 2002; Loftsson, Jarho, Masson, & Jarvinen, 2005). Some investigators, however, have suggested that HPCD may extract the SC lipids and disrupt this barrier layer of the skin (Bentley, Vianna, Wilson, & Collett, 1997). The objective of this study was to investigate the mechanism by which HPCD increases transdermal penetration of drug substances. The study design consisted of two phases: pretreatment of hairless mouse skin with HPCD solutions for up to 4 h, followed by application of corticosteroid-containing suspensions and assessment of transdermal flux and skin accumulation of the model drugs, hydrocortisone (HC) and triamcinolone (TC). Endothermic melting transitions of the SC following pretreatment were also determined as an indication of HPCD-induced lipid disorganization.

MATERIALS AND METHODS

Materials

HC was purchased from Sigma-Aldrich (St. Louis, MO, USA) whereas MP Biomedicals Inc. (Irvine, CA, USA) supplied TC. HPCD (0.8 molar substitution), aqueous formaldehyde solution (37%), trypsin 10× solution, and Brij 58 (polyoxyethylene [20] cetyl ether) were purchased from Sigma-Aldrich. Propylene glycol (PG) was purchased from Spectrum Chemical (Gardena, CA, USA). High-performance liquid chromatography (HPLC)-grade methanol was obtained from Burdick & Jackson (Muskegon, MI, USA).

Preparation of HPCD Solutions and Drug Suspensions

Simple aqueous-based solutions of HPCD (5, 10, and 20%, wt/wt) were prepared by conventional techniques. The appropriate amount of HPCD was mixed with deionized water using a magnetic stir bar until a solution was formed. These HPCD solutions were used as pretreatments prior to application of the corticosteroids. The control was defined as 0% HPCD (deionized water).

Suspensions containing HC or TC were prepared by dissolving excess drug in a phosphate buffer: PG solution (4:1), covering with parafilm, and mixing overnight. PG was used as a constituent solvent because it greatly increased the solubility of the corticosteroids in the suspensions. This concentration of PG was significantly lower than what may be associated with skin barrier disruption, and therefore the possible effects on the flux of the corticosteroids would be exclusively the result of HPCD treatment (Moser, Kriwet, Froehlich, Kalia, & Guy, 2001). The excess drug present in the formulations was used to maintain a constant driving force for the transdermal permeation over the course of the experiment.

In Vitro Transdermal Penetration

In vitro permeability studies were conducted using modified Franz diffusion cells and SKH-1 hairless mouse skin. SKH-1 hairless mouse skin shares the essential structural characteristics with human skin. Although the permeability properties and lipid organization differ from that of human skin, the hairless mouse model was selected for this study because it is commonly employed in transdermal experiments (Kligman, 1996) and the animals are relatively inexpensive and easy to handle. The mice were purchased from Charles River Laboratories (Wilmington, MA, USA). The animal protocol used in this study was approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee. Animals were killed by CO_2 asphyxiation and full-thickness dorsal skin was excised. Any extraneous subcutaneous fat was removed from the dermal surface. Skin samples were then stored at –10°C. Research involving various skin types including human, cattle, and nude rat has demonstrated that freezing prior to experimentation does not alter the barrier transport kinetics of skin (Kastin & Bowman, 1990; Pitman & Downes, 1982). At the time of experimentation, skin samples were thawed and then mounted onto the receptor compartments of modified Franz diffusion cells. Each receptor cell contained isotonic phosphate buffer (pH 7.2), maintained at 37 ± 0.5°C and continuously stirred at 600 rpm with a magnetic stir bar. Brij 58 (0.5, wt/vol) was added as a solubilizer. Following a 1-h equilibration period, 300 µL of the HPCD solutions (up to 20%, wt/wt) was applied to the skin. After a 1- or 4 h pretreatment, the HPCD solution on the skin surface was removed by blotting with lint-free wipes. Next, 300 µL of the HC or TC suspensions was applied. Samples (300 µl) were withdrawn from the receptor compartment at predetermined time points up to 24 h, with the withdrawn volume immediately replaced with fresh buffer solution. Samples were then analyzed using HPLC.

Concentration versus time graphs were constructed for each drug at each HPCD pretreatment, and the slopes were determined using linear regression. Dermal kinetic parameters, including the permeability coefficient (k_p) and flux (rate of skin permeation), were calculated using Equations 1 and 2, respectively, where J is flux in µg/cm^2/h and C_{app} is the concentration of drug in solution applied to the skin.

$$k_p = \frac{\text{receptor cell volume (cm}^3) \times \text{slope of linear portion of time – concentration graph (}\mu\text{g/ h/mL)}}{\text{area of donor compartment (cm}^2) \times \text{model compound concentration (}\mu\text{g/mL)}} \quad (1)$$

$$J = k_p \times C_{app} \quad (2)$$

In Vitro Skin Accumulation

After 1- or 4-h pretreatment with HPCD, 300 µL of the drug-containing suspensions was applied. Initial trials showed HC and TC concentrations in the receptor fluid were low until

the 8 h time point; therefore, the total accumulation of each drug in the mouse skin was determined at 8 h following application. Skin samples were removed from the receptor cells and rinsed with methanol to remove residual drug from the skin surface. Each skin sample was blotted dry, weighed, cut up, placed in 2 mL of methanol, homogenized using a tissue homogenizer (Biospec Product, Racine, WI, USA), and then finally centrifuged at 3,300 rpm (Fisher Benchtop Centrifuge, Fisher, Pittsburgh, PA, USA) for 5 min. The supernatant was analyzed by HPLC for drug concentration.

Analytical Method

Receptor fluid and skin extraction samples were analyzed by HPLC. The liquid chromatograph consisted of a binary pump solvent delivery system (Model P1500, Thermoseparations Products, Riviera Beach, FL, USA), a 100-µl injection loop autosampler (Model AS 1000, Thermoseparations Products), and a variable-wavelength ultraviolet light absorbance detector (Model UV 1000, Thermoseparations Products). The analytical column was a 5 µm pore size, 4.6 mm × 250 mm C_{18} column (Econosphere, Alltech, Deerfield, IL, USA) with a guard column of the same material. The system was controlled and integrated by a personal computer running chromatography management software (PC 1000, Thermoseparations Products). For HC, the detection wavelength was 242 nm, the mobile phase was water:acetaldehyde (70:30) at a flow rate of 1 mL/min, and the retention time was approximately 7 min. For TC, the detection wavelength was 254 nm, the mobile phase contained methanol:150 mM ammonium acetate at a gradient of 40:60 to 50:50 over 6 min at a flow rate of 1 mL/min, and the retention time was approximately 4.5 min.

Determination of Epidermal Lipid Melting Temperatures

The epidermis was separated from full-thickness SKH-1 hairless mouse skin by placing the skin dermis side down on filter paper saturated with 2.5% (wt/vol) trypsin solution. After storage at 37ºC for 4 h, the epidermal sheets were gently removed from the skin using forceps and then covered with fresh trypsin solution and stored at 37ºC for 1 h. The remaining nucleated cells were removed from the epidermis by rinsing with deionized water. The resultant samples were then dried overnight in a 25% relative humidity desiccator. Dried epidermal samples were soaked for 4 h in a 5 mL solution containing 0, 5, 10, or 20% HPCD. Following treatment with HPCD, the samples were blotted dry, weighed, sealed in aluminum pans, and analyzed using a 2920 Modulated Differential Scanning Calorimeter (MDSC, TA Instruments, New Castle, DE, USA). The samples were heated at a rate of 10ºC/min from 0ºC to 180ºC with a temperature modulation of 0.759ºC/min. The SC lipid melting temperatures of the epidermal samples were determined using TA Instruments Universal Analysis Software.

Data Analysis

Statistical analysis was carried out using SigmaStat (SPSS Inc., Chicago, IL, USA). For statistical comparison, a one-way analysis of variance (ANOVA) was used. A $p < .05$ was considered statistically significant.

RESULTS

Influence of HPCD Pretreatment on Transdermal Flux and Skin Accumulation of Model Corticosteroids

The rate of transdermal permeation (flux) of HC and TC after HPCD pretreatment is shown in Figure 1. The flux of HC (Figure 1A) was approximately 10 times greater than TC (Figure 1B), irrespective of HPCD pretreatment. These results indicate that the more lipophilic steroids such as HC (with log *P* value of 1.55) are able to penetrate through the skin faster than TC (with a log *P* value of 1.03). A one-way ANOVA showed no statistical difference in the flux between the 1 and 4 h of HPCD pretreatment for HC and TC, or between low and high concentrations of HPCD pretreatment for HC and TC.

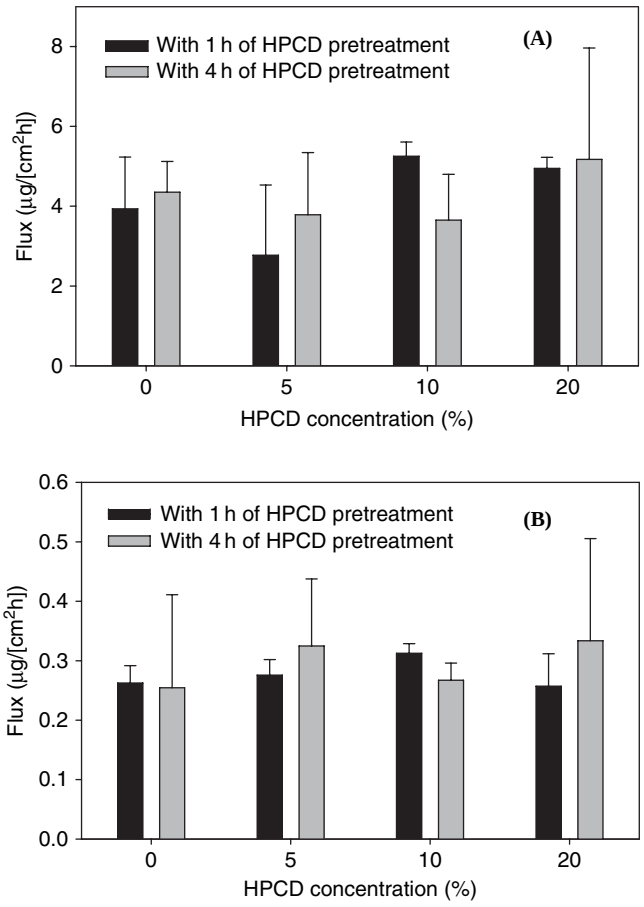

FIGURE 1. Influence of hydroxypropyl-β-cyclodextrin (HPCD) pretreatment on transdermal flux of (A) hydrocortisone (HC) and (B) triamcinolone (TC) ($n = 4$).

These data demonstrate that pretreatment with up to 20% (wt/wt) HPCD for up to 4 h did not affect skin permeability, and thus, the barrier function of the skin remained intact and unaffected by HPCD.

Accumulation of the model corticosteroids in the skin after HPCD pretreatment is shown in Figure 2. As was seen from the permeation data, there was a difference in the total amount of drug accumulated in the skin between the two corticosteroids, with the more lipophilic HC found in concentrations larger than those of TC. For both corticosteroids, a significant difference was detected when comparing the 1-h and 4-h pretreatments of the skin with the control (0% HPCD), suggesting there is a relationship between skin hydration and uptake of the drug into the skin. These findings are in agreement with those of other researchers who showed the water level in the skin could greatly influence the extracellular space (Gloor, Bettinger, & Gehring, 1998; Hikima & Maibach, 2006; Rawlings & Harding, 2004). For HC (Figure 2A), a trend of slightly increasing drug accumulation with increased HPCD concentration from 5 to 20% was noted; however, the difference did not achieve statistical significance. For TC (Figure 2B), no significant difference was detected when comparing the accumulation data between different concentrations of HPCD pretreatments, regardless of pretreatment time.

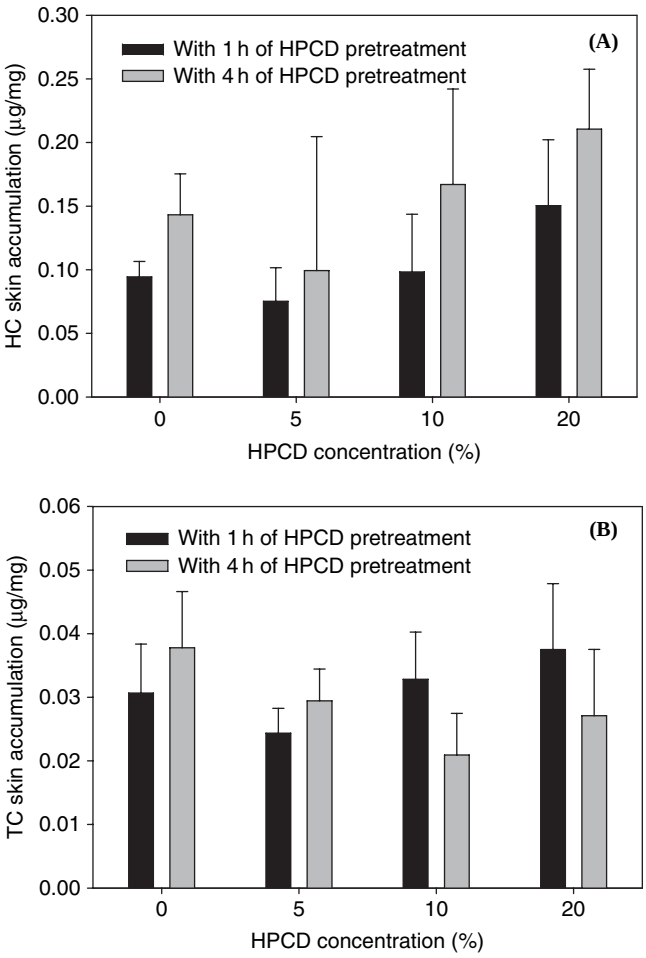

FIGURE 2. Influence of hydroxypropyl-β-cyclodextrin (HPCD) pretreatment on skin accumulation of (A) hydrocortisone (HC) and (B) triamcinolone (TC) (n = 4).

Influence of HPCD Treatment on the Epidermal Lipid Melting Temperatures

Differential scanning calorimetry (DSC) is a convenient and sensitive method for studying the structural properties of the epidermis. An increase in the epidermal structural disorder has been correlated with a decrease in epidermal lipid melting temperature (Bentley et al., 1997; Felton, Wiley, & Godwin, 2004). In this study, DSC was used to investigate the SC lipid/protein phase transitions after HPCD treatment. The control sample (skins with 0% HPCD treatment) exhibited three major endothermic transition peaks, around 67.8, 94.4, and 125.2ºC. The 67.8ºC (T_1) transition indicates disruption of the SC intercellular lipid structure; the 94.4ºC (T_2) transition is ascribed to the break up of the SC protein–lipid interaction, and the 125.2ºC (T_3) transition results from SC protein denaturation (Vollmer et al., 1994). Because the T_2 transition was small or nondetectable in most thermographs, only T_1 and T_3 were determined in this study.

As shown in Figure 3, skin samples exhibited a slight but insignificant decrease in the T_1 transition as the concentration of HPCD increased (T_1 of 5, 10, and 20% HPCD-treated skin was 66.8, 63.5, and 58.2ºC, respectively), suggesting that pretreatment with up to 20% HPCD did not significantly alter the SC lipid organization. A similar downward trend was noted with increasing HPCD concentrations for the T_3 transition (T_3 of 5, 10, and 20% HPCD-treated skin was 129.9, 127.3, and 114.6ºC, respectively). A significantly lower T_3 was detected from the epidermis sample exposed to the 20% HPCD compared with the control (0% HPCD) ($p < .05$), indicating that the protein structure of the SC was disrupted when exposed to high HPCD concentration (20%, wt/wt) for 4 h. This alteration could be attributed to a hydrogen-bonding interaction between the hydroxy group of HPCD and the hydrogen-donating keratins, which are the primary protein components in the SC. This interaction, however, did not affect the transdermal penetration of the corticosteroids that presumably penetrated through the lipophilic intercellular channels.

DISCUSSION

Cyclodextrin complexation has been used to alter the physicochemical properties of various drugs (Challa, Ahuja, Ali, & Khar, 2005; Felton et al., 2004; Loftsson & Masson, 2001), and this technology has been applied to topical formulations to increase drug permeation (Babu & Pandit, 2004; Felton et al., 2002;Williams, Shatri, & Barry, 1998). There is, however, controversy about how cyclodextrins

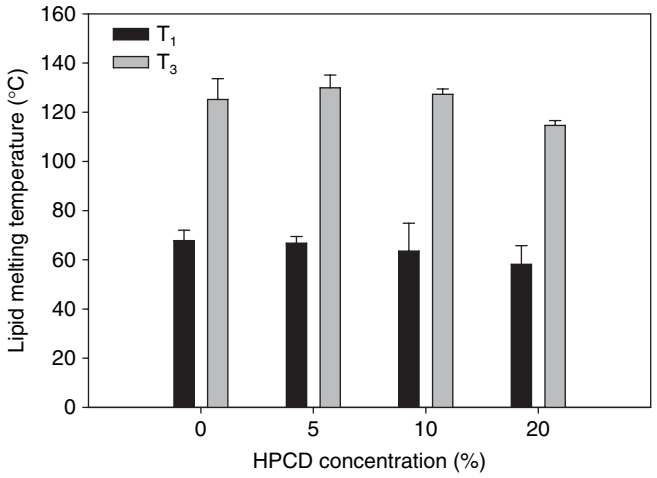

FIGURE 3. Influence of hydroxypropyl-β-cyclodextrin (HPCD) treatment on epidermal endothermic melting transitions (n = 3).

function as penetration enhancers. Some researchers have theorized that cyclodextrins enhance the apparent aqueous solubility of drugs (Loftsson et al., 1994), whereas others have suggested that these excipients complex lipophilic materials in the SC to disrupt its barrier function (Bentley et al., 1997; Legendre et al., 1995). Further complicating the question, still others have shown that cyclodextrin complexation can either increase or decrease transdermal penetration, depending on the concentration of the cyclodextrin used. Increasing concentrations of cyclodextrin, up to that necessary to solubilize the drug in the formulation, has been shown to enhance drug penetration (Felton et al., 2002; Loftsson & Masson, 2001).

In this study, skin was first exposed to HPCD solutions as a "pretreatment," thus allowing the effect of the cyclodextrin on skin permeability to be assessed, rather than determining the influence of complexation on aqueous solubility. We found that skin permeability was not affected by HPCD pretreatment, suggesting that the barrier function of the skin remained intact. Furthermore, the SC lipid organization was not disrupted by HPCD, as indicated by the DSC data. These results are in agreement with those of Williams et al., who showed cyclodextrin pretreatment of human skin did not produce an increase in transdermal flux (Williams et al., 1998). However, a previously published study suggested that HPCD extracted epidermal lipids, which disrupted the skin barrier function and resulted in increased drug penetration (Bentley et al., 1997). This earlier study used a time-consuming separation technique followed by a 12-h incubation period with HPCD prior to DSC analysis, and thus, the observed alterations in the SC lipid melting transitions were likely a result of long-term hydration-induced epidermal disorganization.

CONCLUSIONS

In this study, pretreatment of skin with up to 20% (wt/wt) HPCD did not significantly alter transdermal flux and skin accumulation of the model corticosteroids, suggesting that the SC barrier properties remained intact under these experimental conditions. These results are supported by DSC examination of the SC lipids structure, which showed no obvious evidence of HPCD-induced lipid disorganization such as lipid extraction or altered state of lipid fluidization. DSC results, however, suggest that high HPCD concentrations can disrupt the protein structure of the SC, which may impact transdermal penetration of more hydrophilic compounds. These data support the hypothesis that the penetration enhancement properties of cyclodextrins are due to an increase in the apparent aqueous solubility of the drug and hence the thermodynamic driving force for transdermal permeation rather than extracting lipids from the skin.

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