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A protocol for the assessment of receiver solution additive-induced skin permeability changes. An example with γ-cyclodextrin

Joseph Sclafani [a,*], Puchun Liu [a], Erica Hansen [a], Melinda G. Cettina [b], Jim Nightingale [c]

[a] *MR2, Basic Pharmaceutics Research, CIBA, Ardsley, NY 10502, USA*
[b] *Sterling Health, 115 Campus Drive, Princeton, NJ 08540, USA*
[c] *Bend Research Inc., 64550 Research Rd., Bend, OR 97701 8599, USA*

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Abstract

In the in vitro evaluation of transdermal drug delivery, the receiver solution composition can affect the experimental outcome. In particular, receiver solution accumulation of permeants having low aqueous solubility may give misleading results. Additives can be used to increase the solubility in the receiver solution, but have the potential of altering the skin barrier function. We offer a protocol for the evaluation of receiver solution additives with respect to potential detrimental effects on skin permeation, using bovine serum albumin as a control additive and examining the effect of additive concentration. A test receiver solution additive, γ-cyclodextrin, has been effectively used in vitro to address limited receiver solution solubility in the evaluation of transdermal delivery of a model permeant, progesterone. At the cyclodextrin concentrations used, flux experiments detected no alterations in the barrier function of skin.

Keywords: Permeability alteration; Skin barrier function; Receiver solution additive; In vitro transdermal methodology

1. Introduction

During in vitro evaluation of transdermal drug delivery, a permeant (e.g., a drug) transverses the skin and enters a receiver solution. The permeant concentration in the receiver solution is used to calculate a delivery profile. The receiver solution composition (e.g., ionic strength, pH/buffers, additives, etc.) can affect the outcome of these experiments (Bronaugh and Stewart, 1984; Collier and Bronaugh, 1991; Kou et al., 1993). In particular, accumulation of permeants having low aqueous solubility in the receiver solution may give misleading results, as the concentration there can exceed sink conditions, causing the flux to decrease. This motivates the study of additives to

* Corresponding author.

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maintain an elevated receiver solution solubility of the permeant under investigation, while not altering the barrier function of the skin. These additives must be carefully chosen, because receiver solution induced permeability changes are to be avoided. In this work, we establish an experimental protocol to screen receiver solution additives (RSA) for RSA-induced skin permeation changes by comparison of permeant fluxes in the presence of a test-RSA with those realized using a control RSA, and by examining the effect of the RSA concentration.

For our control RSA, we chose bovine serum albumin (BSA), which is often used as an RSA in transdermal transport experiments to combat low receiver solution solubility and to mimic physiological conditions (Bronaugh and Stewart, 1984; Pozzo et al., 1991; Brown and Ulsamer, 1975). Progesterone, a widely studied lipophilic compound, was used as model permeant having low water solubility (Marty et al., 1980). Finally, the test-RSA, γ-cyclodextrin (CD), was selected from among the α, ß and γ-CDs, because γ-CD alone, gave significantly improved progesterone solubility in receiver solution. These solubility data are reported.

Two types of experiments were run to assess γ-CDs affect on skin permeability. First, transport experiments with γ-CDs in receiver solution are compared to CD-free receiver solutions containing 3% BSA. Second, the transdermal flux of progesterone was measured with γ-CD present at the target concentration in the receiver solutions and with various concentrations of γ-CDs present in the donor solutions.

2. Materials and methods

2.1. Materials

Progesterone, BSA (98–99% albumin), gentamicin sulfate and the α, β, and γ-cyclodextrins were used as obtained from Sigma Chemical Co. (St. Louis, MO). Materials for HPLC and ethyl acetate were all of HPLC grade. All other chemicals were reagent grade. Solutions were prepared with 18 MΩ cm purified water.

2.2. Assay methods

Progesterone was quantified by HPLC on a 3 cm Perkin-Elmer ODS column with a mobile phase of 60:40 (v/v) acetonitrile/water flowing at 1.5 ml/min. UV detection at 240 nm gave a retention time of 1.6 min with no significant chromatographic interference between CD and progesterone.

2.3. CD and BSA solutions

100 μl of CD solutions were directly injected on the HPLC. 3 ml of BSA solution was extracted with 6 ml of ethyl acetate by mixing for 20 min on a Laboratory Rotator (Glas-Col Inc.) at a setting of 3 and then centrifuged for 15 min at 2000 rpm at room temperature. 1 ml of the organic upper phase was transferred to an HPLC vial and dried under a stream of N_2. After resuspension and mixing of the residue in 1 ml of the mobile phase, 100 μl was injected on the HPLC.

2.4. Progesterone solubilities

Excess progesterone was equilibrated at room temperature (25 ± 1°C) in a stirred receiver solution containing a known concentration (% w/v) of either CD or 3% BSA. Samples were centrifuged for 20 min at 2000 rpm at room temperature. The supernatants were diluted and quantified by HPLC. Solubilities were examined for 2 weeks to ensure equilibrium.

2.5. Permeation experiments

Vertically assembled LGA cells (Laboratory Glass Apparatus Inc., Berkeley, CA) with a diffusional area of 3.3 cm^2 and a receiver chamber volume of approx. 5 ml were maintained at 32°C. Each receiver chamber content was stirred at 600 rpm with a small teflon coated magnetic stirrer. The receiver solution consisted of normal saline (0.9% w/v NaCl) containing 0.01% (w/v) gentamicin sulfate and the appropriate CD or BSA concentration (% w/v). BSA receiver solutions were filtered through a 5 μm filter under pressure to ensure particle-free solutions. A peri-

staltic pump provided continuous flow at 6–7 ml/h and samples were collected hourly into tared test tubes using a fraction collector. Intact epidermal membrane was isolated from dermatomed human cadaver skin of transplantable quality that had been stored at −70°C (Kligman and Christophers, 1963). The 5 ml donor solutions, saturated with progesterone, had a slight excess of crystals to ensure unit activity and infinite dose donors. This was applied to the stratum corneum side of skin clamped in an LGA cell. Since permeation profiles can be significantly affected by flow-through apparatus variables, the receiver volume, flow rate, diffusional area, and fraction collector interval were all held constant (Sclafani et al., 1993).

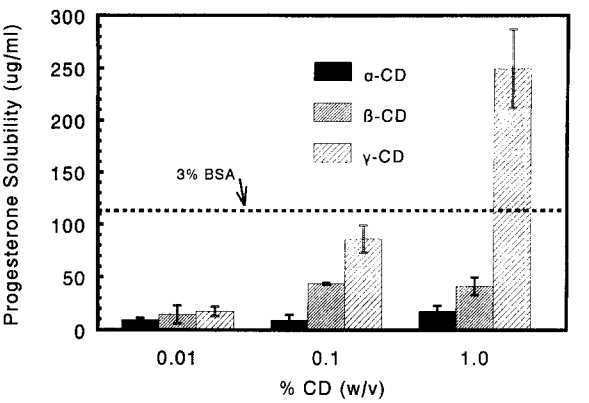

Fig. 1. Solubility of progesterone in receiver solutions containing either CD, at the indicated concentration, or 3% BSA. The error bars are the standard deviation of three replicates. BSA had a standard deviation of ±6 μg/ml.

3. Results

The solubility of progesterone was measured in receiver solutions containing one of the three CDs at different concentrations or in the control receiver solution with 3% BSA. CDs were selected as test-RSAs because they can be directly injected on the HPLC. This contrasts HPLC quantification of solutions containing BSA, where the permeant must first be separated from the BSA via a laborious extraction process. The results, shown in Fig. 1, indicate that only γ-CD increases the progesterone solubility to a significant extent, suggesting that of the three CDs, only γ-CD would be useful as a receiver solution additive. The solubility enhancement with γ-CD, relative to the other CDs, is probably due to the larger size of the hydrophobic cavity (Uekama et al., 1982). These results are consistent with the findings of Uekama et al. (1982) who performed similar progesterone solubility measurements for γ-CD in water without NaCl or gentamicin sulfate. The progesterone solubility in γ-CD or BSA receiver solutions represents a substantial increase over the reported value of 12 μg/ml in water alone (Florence, 1981). Therefore, flux measurements were performed with either γ-CD or BSA-control in the receiver solutions. The target concentration of 0.1% γ-CD in the receiver solution was chosen so as to approach the solubility of progesterone in the 3% BSA-control receiver solution.

Fig. 2 presents progesterone fluxes from a series of experiments which compares receiver solutions containing either γ-CD or BSA. Progesterone fluxes from CD-free donor solutions into receiver solutions containing 0.1% γ-CD were

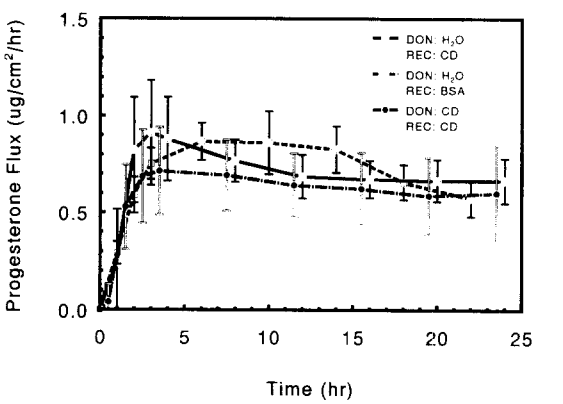

Fig. 2. Comparison of (i) γ-CD or BSA in the receiver solution and (ii) CD or CD-free donors with CD in the receiver solution. Open symbols indicate 'asymmetric' case; fluxes from CD-free donors (DON) into receiver solution containing either 0.1% γ-CD or 3% BSA (REC). The solid symbols indicate 'symmetric' case; flux from donors containing 0.1% γ-CD into receiver solution containing 0.1% γ-CD. The error bars are the standard deviation for four replicates, except for three replicates for the BSA receiver. Skin donor no. 1142; 51 year old female.

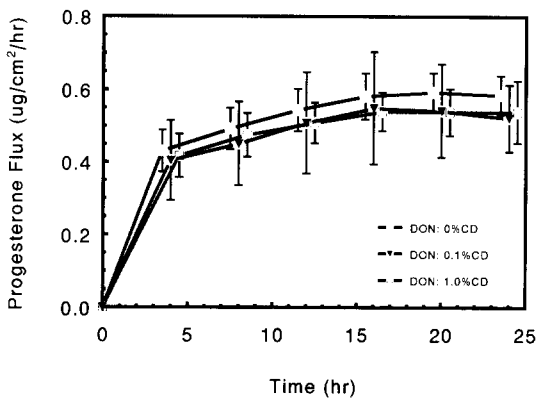

Fig. 3. Effect of increasing γ-CD concentration in the donor solution (DON) on progesterone flux into receiver solution containing 0.1% γ-CD. Open and solid symbols indicate asymmetric and symmetric CD concentrations, respectively. The error bars are the standard deviation for three replicates, except for four replicates for the 1%CD donor. Skin donor no. 1787; 53 year old male.

compared to the 3% BSA-control receiver solutions. These CD-free donors paired with CD-containing receiver solutions represent 'asymmetric' CD configurations across the skin. In contrast, the 'symmetric' set-up has 0.1% γ-CD on both the donor and receiver sides of the skin, the donor again being saturated with progesterone. The motivation for this symmetric experiment is to assess whether γ-CD in intimate contact with the stratum corneum would participate in stratum corneum alterations that would increase the progesterone flux relative to the asymmetric γ-CD arrangement. As seen in Fig. 2, the flux profiles for each of these runs demonstrate no significant statistical difference (*t*-test). The data in Fig. 2 suggest that there is no alteration of overall skin barrier function.

The dependence of skin barrier function on γ-CD concentration was probed in a series of experiments on a new skin donor (Fig. 3). In each of these experiments, the receiver solution contained 0.1% γ-CD. The concentration of γ-CD in the progesterone-saturated donor solution was varied (0, 0.1 and 1%). This arrangement avoids possible diffusional lag times which may be associated with the epidermis. No significant statistical difference in progesterone flux was observed (*t*-test) as the donor-side CD concentration was increased, even up to 1% γ-CD. These data demonstrate that even these fairly elevated γ-CD concentrations do not alter the barrier properties of skin to progesterone.

4. Discussion

In our example, γ-CD, as a RSA at 0.1%, was found to have no effect on the barrier function of skin. Recently, hydroxypropyl-β-CD (HPCD) has been reported to act as flux enhancers for transdermal permeation (Loftsson and Bodor, 1989; Vollmer et al., 1993). HPCD is a CD which is chemically modified to increase its solubility in aqueous media. Therefore, this might be taken to imply that CDs in general would alter skin permeability. However, these studies, in which flux enhancement was reported, used concentrations in excess of 20% (w/v) in the donor chamber. Results with HPCDs at lower concentrations support our findings of no flux enhancement and demonstrate the importance of additive concentration. For example, Vollmer et al. (1993) report their lowest HPCD concentration of 4.9% to be ineffective as a skin permeation enhancer. γ-CD has a molecular weight of 1300 and a large radius because of its ring-like molecular geometry. Such molecules are unlikely to permeate the stratum corneum as compared to smaller, less bulky compounds (Potts and Guy, 1992). These considerations favor our finding of no changes in skin permeability or skin barrier function.

In contrast to our results with the relatively large γ-CD, smaller compounds that have been used as RSAs in in vitro transdermal studies have been found to influence the barrier function of skin. For example, alkanols have been studied in receiver solutions and found to affect the stratum corneum and enhance permeant fluxes (Collier and Bronaugh, 1991; Kim et al., 1992). Therefore, at the concentrations reported in these studies, these solvents should not be used as RSAs due to their effect on skin flux enhancement. Before a transdermal permeation study is undertaken in the presence of RSAs, their effect on the skin barrier function must be assessed.

5. Conclusion

We have established a simple experimental protocol to assess receiver solution additive-induced changes in skin permeability and have demonstrated it experimentally. The protocol is summarized below:

- Measure the transdermal flux from a donor solution containing a drug or model permeant (e.g., progesterone) with the RSA under evaluation (e.g., γ-CD) in the receiver solution at the target concentration.
- Compare this flux to that realized in the presence of a control-RSA (e.g., BSA).
- Investigate the potential of a concentration effect due to the RSA, by comparing the results of diffusion experiments where RSA is added to the donor solution at varying concentrations (e.g., 0, 0.1 and 1% γ-CD) with the test-RSA in the receiver fixed at the target concentration.

In our example, γ-CD, as an RSA at 0.1%, was found to have no effect on the barrier function of skin.

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