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In vitro experiment optimization for measuring tetrahydrocannabinol skin permeation

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Abstract

The purpose of this study was to optimize in vitro experimental conditions for the measurement of Δ^9-tetrahydrocannabinol (Δ^9-THC) permeation across human skin using a flow-through diffusion cell system. The drug permeation rates through intact and stripped (stratum corneum (SC) removed) skin were also compared in order to determine if the SC provided significant resistance to the diffusion of hydrophobic Δ^9-THC. The receiver fluids evaluated were HEPES-buffered Hank's balanced salt solution (HHBSS) with either 4 or 6% bovine serum albumin (BSA), Polyoxyethylene 20 Oleyl Ether (Brij 98) solution (0.5 and 6.0%), and hydroxypropyl-β-cyclodextrin (HPBCD). The Δ^9-THC permeability was significantly higher into Brij 98 solutions than into 4% BSA. BSA 6% receiver solutions showed significantly higher Δ^9-THC permeation over BSA 4%. There were no significant differences in Δ^9-THC permeability or lag time values between 0.5 and 6% Brij 98 receiver solutions. HPBCD failed to work as a suitable receiver solution. The Δ^9-THC flux in the stripped skin experiments exceeded the flux in the intact skin experiments. It appears that the SC provides some resistance to the diffusion of Δ^9-THC across human skin. These experimental results have confirmed the utility of several receiver solutions for the in vitro human skin diffusion study of Δ^9-THC.

Keywords: Tetrahydrocannabinol; THC; Transdermal delivery; Human skin; Percutaneous permeation; In vitro diffusion

1. Introduction

Dronabinol (Marinol®) is Δ^9-tetrahydrocannabinol (Δ^9-THC) in an oral capsule form. This drug is prescribed in order to increase appetite and weight gain in AIDS and cancer patients; as well as to alleviate the nausea and vomiting side effects associated with cancer chemotherapy (PDR, 1996). However, some severely nauseated patients may not be able to keep the capsules in their stomachs long enough for the drug to take effect. There is a need for an alternate non-oral dosage form of dronabinol. Additionally, more patients would benefit from the therapeutic effects of Δ^9-THC, if they could tolerate the psychoactive and other central nervous system side effects of dronabinol capsules or smoked marijuana (Vinciguerra et al., 1988). Transdermal Δ^9-THC delivery may be the therapeutic answer for severely

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nauseated patients and those with dose-related side effects. One of the major therapeutic advantages that transdermal delivery has over multiple dosing (oral or smoke inhalation) is the elimination of peaks and troughs in drug plasma concentrations. The zero-order delivery that transdermal systems provide helps to keep therapeutic drug levels constant, obviating the peak plasma levels that often cause increased side effects.

The skin consists of two major layers, the outer epidermis and the inner dermis. The non-viable stratum corneum (SC), the outermost 10–20 μm of the epidermis, is responsible for the skin's tremendous diffusional resistance to the transdermal delivery of many drugs. The skin vasculature is supported by the dermis and lies a few microns underneath the epidermis. In contrast to SC-controlled drug diffusion, the extreme lipophilicity of Δ^9-THC may make traversing the aqueous media of the skin's viable tissue the rate-limiting step in this drug's diffusion process (Scheuplein and Blank, 1973). In fact, use of full-thickness skin in diffusion studies may provide a significant underestimate of the actual in vivo transdermal flux, because of the potential for lipophilic compounds like Δ^9-THC to diffuse slowly through the aqueous environment of the dermis (Bronaugh, 1996).

In vitro human skin studies to measure drug diffusion and metabolism provide the most accurate absorption data, as compared with animal skin studies. It is very important that the in vitro model studies simulate in vivo conditions, including maintenance of tissue viability so that any significant cutaneous drug metabolism can be observed (Collier et al., 1991). Thus, selection of an ideal receiver solution is crucial to the success of these in vitro studies. The receiver solution helps to maintain not only tissue viability, but sink conditions in the experiment as well. Hydrophobic drugs like Δ^9-THC must be soluble enough in the receiver solutions so that sink conditions are maintained (Bronaugh and Stewart, 1984). Therefore, the present study focused on optimization of in vitro experimental conditions for the measurement of Δ^9-THC across human skin. Additionally, intact and stripped (SC removed) skin were compared in order to determine if the SC provided significant resistance to the diffusion of highly lipophilic Δ^9-THC.

2. Materials and methods

2.1. Chemicals

Δ^9-THC in 95% ethyl alcohol was obtained in ampoules from the National Institute on Drug Abuse (NIDA, Research Triangle Park, NC). Hank's balanced salts modified powder, bovine albumin fraction V (BSA), potassium phosphate monobasic anhydrous, sodium bicarbonate, and Polyoxyethylene 20 Oleyl Ether (Brij 98®) were obtained from Sigma (St. Louis, MO). Propylene glycol, 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), triethylamine (TEA), gentamicin sulfate, and acetonitrile (HPLC grade) were obtained from Fisher Scientific (Fairlawn, NJ). 2-Hydroxypropyl-β-cyclodextrin (HPBCD) was obtained from RBI (Natick, MA).

2.2. Instruments

Equipment used consisted of PermeGear® flow through diffusion cells of area 0.95 cm^2 (PermeGear, Riegelsville, PA), Water Bath 280 series and Shallow Form Shaker Bath (Precision, Winchester, VA), Isotemp 2006S water circulator (Fisher Scientific, Fairlawn, NJ), Retriever IV Fraction collector (ISCO Inc., Lincoln, NE), Pumppro® MPL Static pump (Watson Marlow, Wilmington, MA), Sartorius BP211D model balance (Sartorius, Edgewood, NY), Padgett Dermatome (Padgett Instruments, Kansas City, MO), and an HPLC with a 200 series autosampler and UV detector model 785A (Perkin–Elmer, CT).

2.3. In vitro diffusion studies

2.3.1. Receiver solutions

2.3.1.1. HHBSS with BSA. HEPES buffered-Hank's balanced salt solution (HHBSS) was prepared and filtered. Fifty μg/ml of gentamicin sulfate was dissolved in the receiver solution to minimize microbial contamination. Appropriate

amounts of bovine serum albumin (4 or 6%) were added to the HHBSS solutions. All glassware used was sterilized with 70% v/v ethanol.

2.3.1.2. BRIJ 98 and HPBCD solutions. Appropriate amounts of Brij 98 (0.5 and 6% w/v) and HPBCD (1.4% w/v) were weighed and dissolved in 1000 ml of filtered distilled water to obtain the necessary receiver solutions.

2.3.2. Human skin preparation

Human skin samples from abdominoplasty surgery were obtained from the National Cancer Institute's Cooperative Human Tissue Network (CHTN). The samples were dermatomed immediately upon arrival to a thickness of approximately 200 μm. The actual skin thickness was measured with a micrometer. The samples were either used immediately or frozen at −20 °C. The stripped skin samples were obtained by removing the SC with Scotch® book tape Number 845. The tape strip number varied from 10 to 30, in order to get as much SC removal as possible from the different skin samples. SC removal was confirmed by visualization of a shiny surface (Kammerau et al., 1975; Tsai et al., 1991).

2.3.3. Δ^9-THC formulation

The drug formulation consisted of 8.59 mg/ml Δ^9-THC in propylene glycol:water:ethanol (9:1:0.4).

2.3.4. In-vitro experimental conditions

The temperature of the diffusion cells was maintained at 32 °C with a circulating water bath. The diffusion cells were sterilized with 70% v/v ethanol before securing the skin samples into the cell. The diffusion experiment was initiated by charging the donor compartment with 0.24 ml of drug solution. Each donor cell was capped for the duration of the experiment. The receiver solution was pumped through the diffusion cells at a flow rate of 1.1 ml/h for either 48 or 96 h. Samples were collected with a fraction collector at 6 h intervals. The diffusion samples were refrigerated until analysis. At the end of the stripped versus intact skin experiment, the treated skin area was excised from the skin sample in order to measure tissue THC concentrations. The formulation was washed off of the skin, and the weighed tissue was placed in acetonitrile to shake at room temperature overnight. The extracted THC was then quantitated by HPLC analysis.

2.3.5. Brij 98 pretreatment studies

The receiver solution for 48 h was Brij 98, followed by 48 h of BSA (4%). Brij 98 and BSA (4%) control experiments were run for 96 h.

2.4. Sample preparation

2.4.1. BSA samples

For drug extraction from the BSA diffusion samples, a 4-fold volume of acetonitrile (ACN) was added to each sample. The sample was vortexed for 1 min, sonicated for 15 min, and vortexed for an additional 1 min followed by centrifugation at $5800 \times g$ for 15 min. The supernatant was transferred to silanized autosampler vials and 100 μl of each sample was injected onto the HPLC column. Recovery was 90%.

2.4.2. BRIJ 98 samples

The diffusion samples collected in Brij 98 (0.5 and 6%) were either directly injected or diluted 1:3 with ACN. A 100 μl sample was injected onto the HPLC column. Drug recovery was 100%.

2.4.3. HPBCD samples

The HPBCD diffusion samples were diluted 1:2 with ACN and vortexed for 1 min. A 100 μl sample was injected onto the HPLC column. Drug recovery was 100%.

2.5. HPLC assay

The mobile phase consisted of (80:20) ACN: phosphate buffer (25 mM KH_2PO_4 + 0.1% TEA, pH 3.0) set at a flow rate of 1.5 ml/min. A reversed phase C_8 Column (Brownlee®, 220 × 4.6 mm, Spheri-5) with a guard column (Brownlee®, Reversed phase, C_8, 15 × 3.2 mm, 7 μm particle size) was used in the assay. The assay run time was 7 min, except for the Brij 98 samples, which were run for an additional 7 min at a flow rate of 2.0 ml/min to wash the Brij 98 from the column.

The UV detector was set at a wavelength of 215 nm. The retention time for Δ^9-THC was 4.0 ± 0.1 min. Standard curves were linear within the range of 25–1000 ng/ml and the assay sensitivity was 5 ng/ml.

2.6. Data treatment

The permeation data were plotted as the cumulative amount of drug collected in the receiver compartment as a function of time. The steady-state flux value for a given run was calculated from Fick's First Law of diffusion. The Δ^9-THC permeability coefficients were calculated from the steady state flux and the drug concentration in the vehicle. The statistical analysis of data was completed with the appropriate application of a Student's t-test, a paired t-test, or a One-way ANOVA using SIGMASTAT (SPSS Inc., Chicago, IL).

3. Results and discussion

Conventional in vitro diffusion study receiver solutions, like normal saline and phosphate buffer, are not ideal solutions for the study of drugs with poor aqueous solubility (Bronaugh and Stewart, 1984). In an effort to choose an appropriate receiver solution that will maintain experimental sink conditions with these lipophilic drugs, several researchers have studied various possibilities; including increased receiver flow rates (Crutcher and Maibach, 1969), solutions of ethanol (Bronaugh and Stewart, 1984; Ghanem et al., 1987; Scott and Ramsey, 1987), methanol (Bronaugh and Stewart, 1984), polyethylene glycol 400 (Scott and Ramsey, 1987; Kim et al., 1996), blood (De Lange et al., 1994), serum (Bronaugh and Stewart, 1986; Scott and Ramsey, 1987; Collier et al., 1989), albumin (Brown and Ulsamer, 1975; Bronaugh and Stewart, 1984; Moloney, 1988; De Lange et al., 1994), and surfactants (Bronaugh and Stewart, 1984, 1985, 1986; Scott and Ramsey, 1987; Moloney, 1988). A few reports about the transdermal delivery of Δ^8-tetrahydrocannabinol (Δ^8-THC) exist in the literature (Touitou et al., 1988a; Touitou and Fabin, 1988b; Fabin and Touitou, 1991). These studies describe the delivery of Δ^8-THC across rodent and human skin in vitro with the help of various penetration enhancers. These researchers used a 1:1 ethanol:water mixture as their receiver solution for in vitro studies.

Maintenance of tissue viability and avoidance of skin barrier damage are the complicating requirements involved in identifying a receiver solution that provides good drug solubility for the essential sink conditions in the diffusion experiment. Many solvents and surfactants are capable of leaching lipids from the skin sample and causing a decrease in the barrier integrity. BSA (4%) is a traditional receiver solution used to study the in vitro skin diffusion of lipophilic drugs, and in these experiments it made a good standard for solubility-effect and possible skin damage-effect comparison of the Brij 98 surfactant. It was necessary to compare BSA (4%) and Brij 98 (6%) receiver solutions under our unique experimental conditions, because variability has been reported in the receiver solution results from different species' skin, as well as from the use of different skin thicknesses (Bronaugh and Stewart, 1986). Fig. 1 shows the Δ^9-THC permeability values in skin from five subjects with both BSA (4%) and Brij 98 (6%) receiver solutions. Although a paired t-test did not show a significant difference in this data ($P = 0.06$), individual Student's t-tests on each subject gave P-values < 0.05. The permeability of Δ^9-THC was consistently significantly higher in the presence of Brij 98, as compared with the BSA receiver solution. No significant differences in lag times were observed.

The higher permeability of Δ^9-THC observed with Brij 98 (Fig. 1) could have been due to the possible skin damaging effects of the surfactant. Therefore, to minimize this potential damage, a lower concentration of Brij 98 (0.5%) was also evaluated. The Δ^9-THC permeability data in three subjects with both 6 and 0.5% Brij 98 receiver solutions is shown in Fig. 2. No significant difference in Δ^9-THC permeabilities into the two concentrations of Brij 98 solutions was observed (paired t-test, $P > 0.3$). No significant differences in lag times were observed. It appears that Δ^9-THC may have sufficient solubility in 0.5% Brij

98, so that the higher concentration of Brij 98 (6%) is not required (Brij 98 critical micellar concentration = 0.029%). Using a lower concentration of the Brij 98 surfactant can potentially limit the skin barrier damage that can occur during these experiments. Bronaugh and Stewart (1986) observed increased cortisone permeability with Brij 98 (6%) compared with Brij 98 (0.5%) in 200 μm thick fuzzy rat skin. The increased permeability of the cortisone control into the Brij 98 (6%) solution was reportedly due to skin damage. The human skin used in our experiments is more resistant to damage than the fuzzy rat skin used by Bronaugh and Stewart, and therefore, we observed no difference between the two concentrations.

In order to confirm that the Brij 98 (6%) was not causing permanent damage to the skin barrier used in our experiments, a Brij 98 pretreatment study was designed. These experiments ran for 96 h, the receiver solution for the first 48 h was Brij 98, followed by 48 h of BSA (4%). Additionally, Brij 98 and BSA (4%) control experiments were run for 96 h. The results of the pretreatment studies in three subjects are shown in Fig. 3a. A one-way repeated measures ANOVA showed a significantly (Tukey post-hoc analysis, $P < 0.05$) increased permeability of Δ^9-THC into Brij 98 solutions, as compared with either the BSA controls or the BSA solutions after Brij 98 pretreatment. The drug permeability values of pretreated subjects into BSA (4%) were not significantly different from BSA (4%) controls run for 96 h. Fig. 3b shows the permeation profile of the pretreatment experiment in subject three. All experiments reached and maintained a steady-state flux. It is clear that the pretreatment flux was similar to the Brij 98 control during the first 48 h, but a significant change in flux appears when the receiver solution is changed to BSA at 48 h. The new flux, after the change in receiver solutions to BSA, is parallel to the BSA control (not significantly different, see Fig. 3a). No lag time differences between both receiver solutions were observed. This data shows that no permanent damage has occurred from the Brij 98 solutions, and that the increased permeabilities seen with Brij 98 (6%) are most likely due to the increased

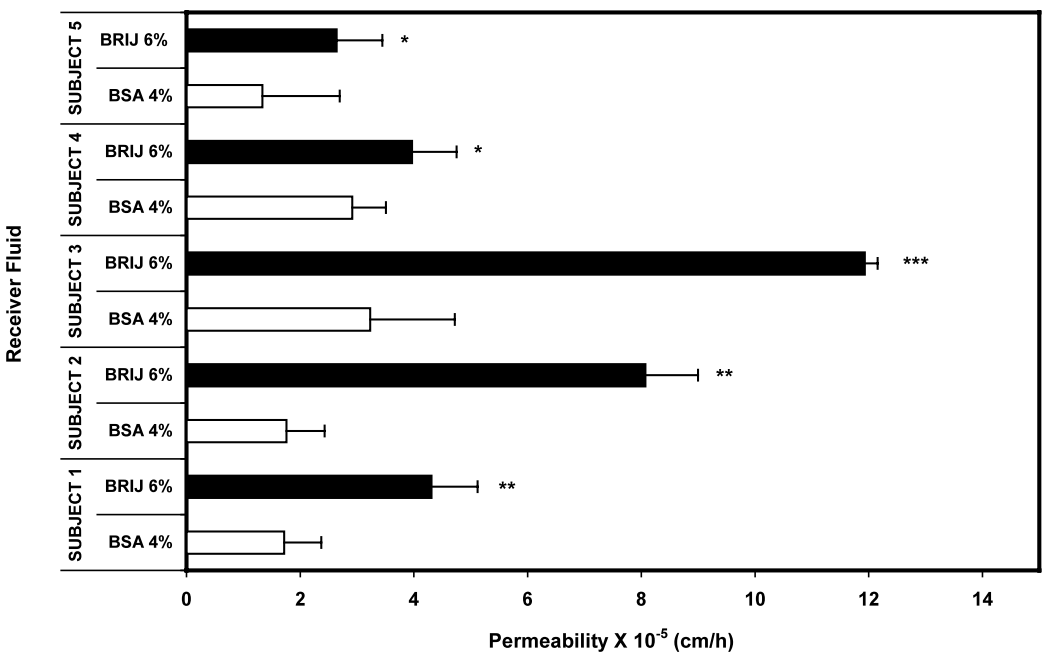


Fig. 1. Human skin permeability of Δ^9-THC: comparative study between BSA (4%) vs. Brij 98 (6%) in five subjects. *, $P < 0.05$. **, $P < 0.01$. ***, $P < 0.001$. Values are the means with standard deviation (S.D.); $n \geq 3$.

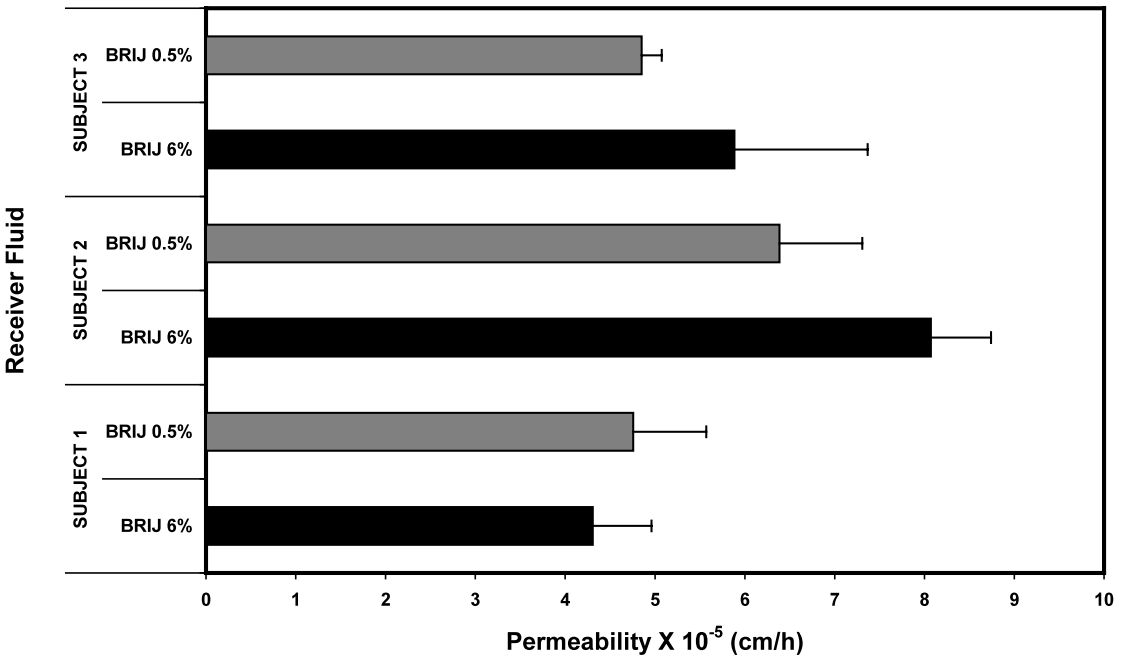
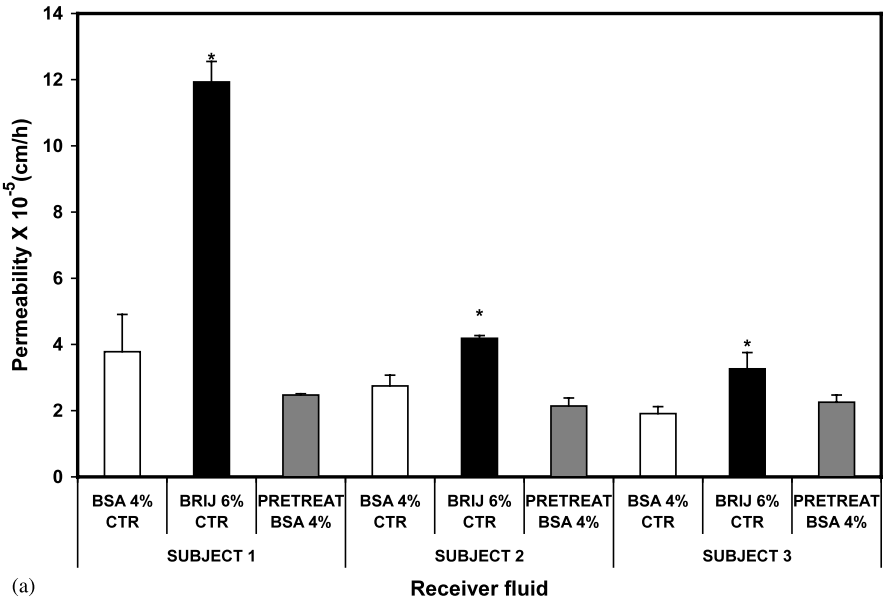


Fig. 2. Human skin permeability of Δ^9-THC: comparative study between Brij 98 0.5 vs. 6% in three subjects. Values are the means with S.D.; $n \geq 3$.

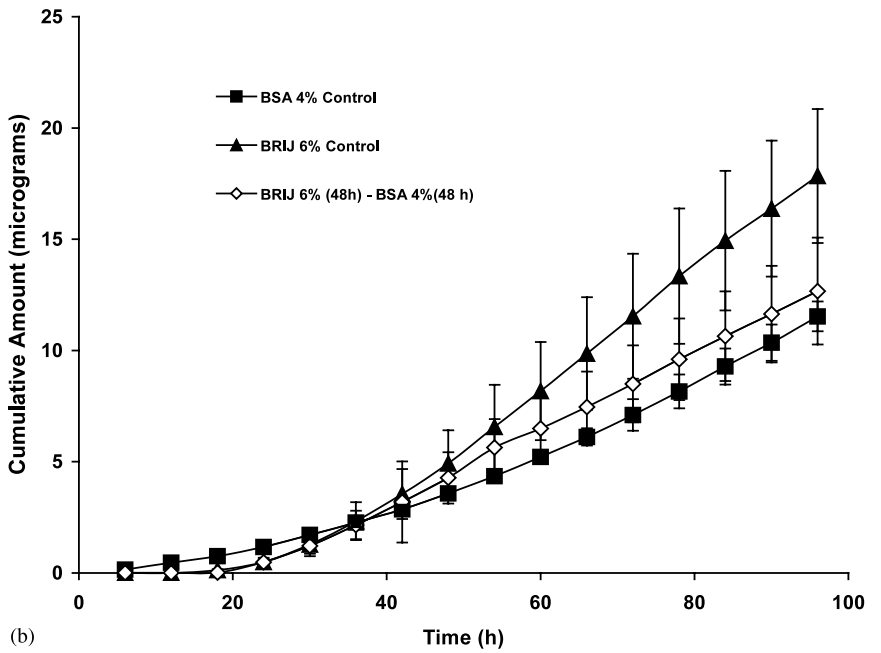
solubility of the Δ^9-THC in Brij 98 (6%) as compared with BSA (4%).

In an effort to increase the Δ^9-THC solubility in the receiver, but also to maintain viability of the skin for cutaneous metabolism studies, BSA (6%) was evaluated. Brij 98 (6%) is not an acceptable receiver solution for use in permeation experiments when observation of cutaneous drug metabolism is a study goal. The results obtained with BSA (6%) in four subjects are shown in Fig. 4. Overall, BSA (6%) proved to be similar in effectiveness as a receiver solution to Brij 98 (6%), and consistently better at solubilizing Δ^9-THC than BSA (4%). A one-way repeated measures ANOVA with Tukey post-hoc analysis showed that the permeability increase of BSA (6%) over BSA (4%) was statistically significant (Tukey post-hoc, $P < 0.01$). The permeability values for BSA (6%) and Brij 98 (6%) were not significantly different. No lag time differences among receiver solutions were observed. BSA (6%) appears to be an acceptable receiver fluid for measurement of Δ^9-THC permeability. As Δ^9-THC formulation studies provide higher levels of drug in the diffusion samples, receiver solutions may need to be adjusted to solubilize higher drug concentrations and maintain sink conditions.

In recent years, cyclodextrins (CD) have been widely used in drug delivery (Loftsson and Brewster, 1996; Rajewski and Stella, 1996), including topical and transdermal dosage forms (Matsuda and Arima, 1999; Loftsson and Masson, 2001). One of the important uses of CD's in drug delivery is to increase the aqueous solubility of lipophilic drugs. The aqueous solubility of Δ^9-THC was enhanced up to 200-fold with HPBCD (Jarho et al., 1998). It has been reported that hydrophilic cyclodextrins and their drug complexes are only able to permeate biological membranes with great difficulty, and only enhance drug permeability by increasing solubility without causing physicochemical changes within the barrier (Loftsson and Masson, 2001). Based on published Δ^9-THC solubility information in HPBCD solutions, an HPBCD concentration of 1% can provide a Δ^9-THC solubility of about 5 μg/ml



(a)



(b)

Fig. 3. (a) Human skin permeability of Δ^9-THC: effect of Brij 98 (6%) pretreatment (48 h) followed by BSA (4%) for 48 h with BSA (4%, 96 h) and Brij 98 (6%, 96 h) controls in three subjects. *, Tukey post-hoc analysis $P < 0.05$ for Brij control vs. BSA control and pretreated BSA. Values are the means with S.D.; $n \geq 3$. (b) Δ^9-THC Permeation Profile in Subject # 3: effect of Brij 98 (6%) pretreatment (48 h) followed by BSA (4%) for 48 h with BSA (4%, 96 h) and Brij 98 (6%, 96 h) controls. Values are the means with S.D.; $n \geq 3$.

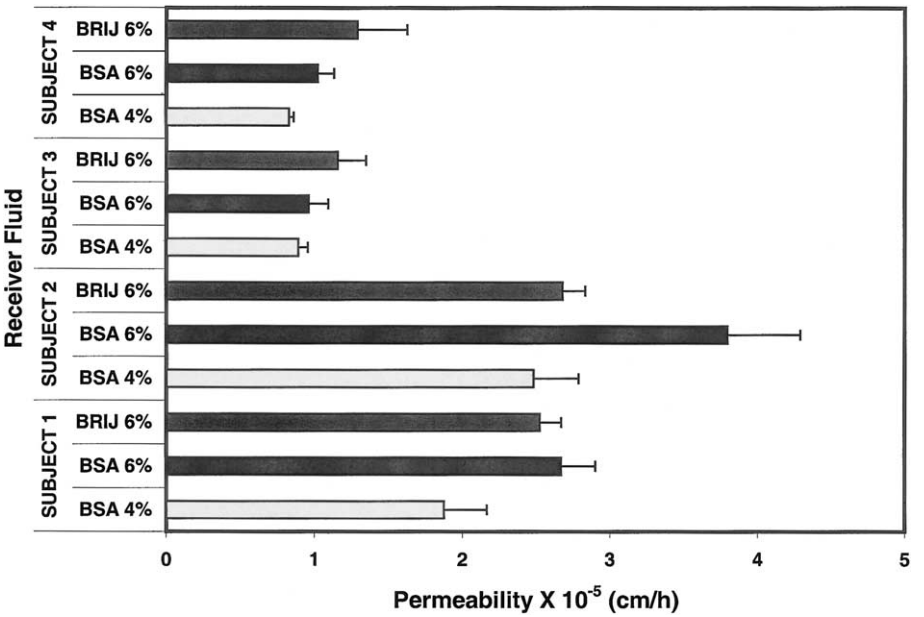


Fig. 4. Human skin permeability of Δ^9-THC: comparative study with BSA (4 vs. 6%) and a Brij 98 (6%) control in four subjects. Values are the means with S.D.; $n \geq 3$.

(Jarho et al., 1998). Typical diffusion samples from the BSA (6%) and Brij 98 (6%) experiments reached a maximum Δ^9-THC concentration of 0.4 μg/ml, more than ten-fold less than the solubility of Δ^9-THC in 1% HPBCD. A 1.4% aqueous solution of HPBCD was used for the initial evaluation of HPBCD as an alternative receiver solution. The results obtained from this study are reported in Fig. 5. The flux value of Δ^9-THC was observed to be the lowest into the HPBCD solution, compared with the other receiver solutions studied. Therefore, it appears that 1.4% HPBCD is not an appropriate receiver solution for Δ^9-THC. Higher concentrations of HPBCD were not studied. HPBCD may not have worked in these experiments for several reasons. It is possible that dermal material was complexed with the HPBCD and decreased the solubilizing capacity of HPBCD. It is also possible that better mixing conditions may be required to solubilize the THC in the HPBCD. There may be a dissolution rate limiting effect for THC in this HPBCD solution.

In the final experiment, the effect of removing the SC was evaluated for its influence on the permeability of Δ^9-THC. Typically, the SC does not provide much of a barrier to hydrophobic drugs, but rather the aqueous layers below the SC provide significant resistance to drug diffusion. Thus, stripped (SC removed) and intact skin were compared using several of the previously studied receiver solutions. The results obtained in five subjects are shown in Fig. 6. A paired t-test showed that the Δ^9-THC permeability in stripped skin is significantly higher than in intact skin ($P < 0.05$). It is clear from the permeability values that a 2–3-fold increase occurs in tape stripped skin as compared with intact skin. The observed mean lag times were found to be significantly shorter for stripped skin (7.8 ± 3.9 h) relative to intact skin (18.0 ± 3.4 h). Mean tissue THC concentrations in the intact skin were almost double that in the stripped skin, when the tissue concentrations were compared on a milligram of THC per gram wet tissue weight basis. As expected, this confirms that the Δ^9-THC exists at a much higher concentration in the SC than the viable tissue layers. The SC does act as a barrier to the permeation of the Δ^9-THC molecule.

It is possible that some of the differences seen between stripped and intact skin in this data are actually underestimated, because of the difficulty of

removing the SC from a hydrated piece of surgical tissue. The tape has some trouble adhering to the damp skin, and this resulted in partial SC removal in some samples. Therefore, the reported 2–3-fold increase in permeability is most likely a minimum level of increase. Overall, the relative Δ^9-THC permeability difference between stripped skin versus intact skin was much less than can be seen with more polar compounds. These results are similar to those found by other researchers who studied the effect of SC removal on various hydrophobic compounds (Feldmann and Maibach, 1965; Hawkins and Reifenrath, 1986). The viable aqueous tissue layers provided a significant amount of resistance to the Δ^9-THC diffusion, as can be seen from the stripped skin experiments.

4. Conclusions

It is equally important to ensure that an appropriate in vitro experimental system is being used to measure percutaneous absorption, as it is to optimize a formulation for the successful transdermal delivery of a drug. An in vitro system that mimics in vivo absorption as closely as possible is the ideal goal. Currently we are performing in vitro/in vivo percutaneous absorption correlation studies in the hairless guinea pig. These in vivo studies will give the best confirmation of our methodology used in the in vitro studies.

From these in vitro human skin diffusion studies, we have concluded that the SC acts as a slight barrier to the permeation of Δ^9-THC. Brij 98 (6%) appears to be a good Δ^9-THC solubilizing receiver solution, as compared with BSA (4%). It appears that Brij 98 in this experimental set-up does not damage the skin and cause increased permeability. Brij (0.5%) may also be an acceptable receiver solution alternative to Brij 98 (6%), as we observed no significant difference between the two solutions. BSA (6%) seems to be a better viability-maintaining receiver solution choice for Δ^9-THC than BSA (4%).

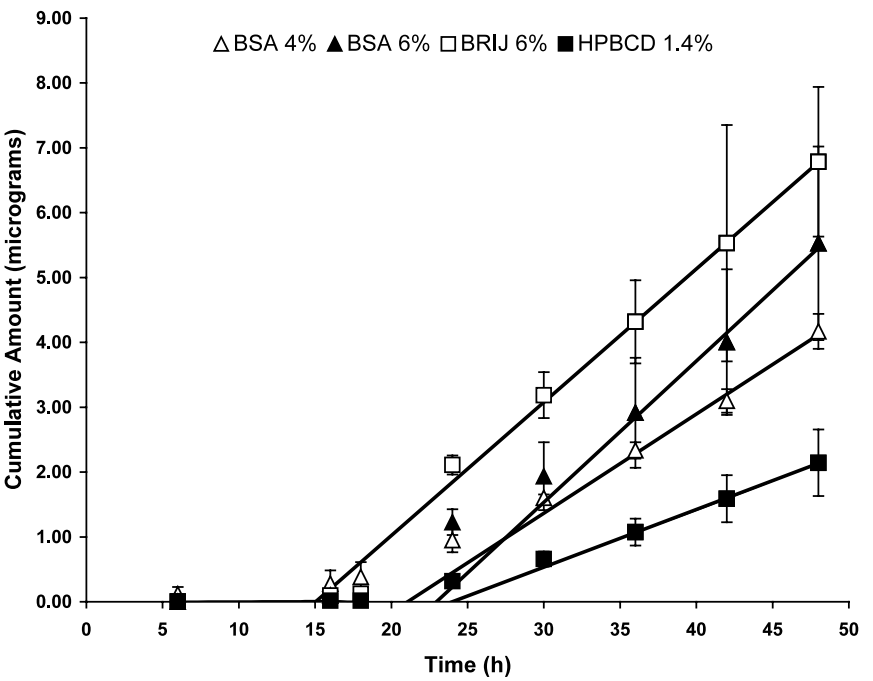


Fig. 5. Δ^9-THC permeation profile in subject # 1: comparative study between Brij 98 (6%), BSA (4 and 6%), and HPBCD (1.4%) as receiver solutions. Values are the means with S.D.; $n \geq 3$.

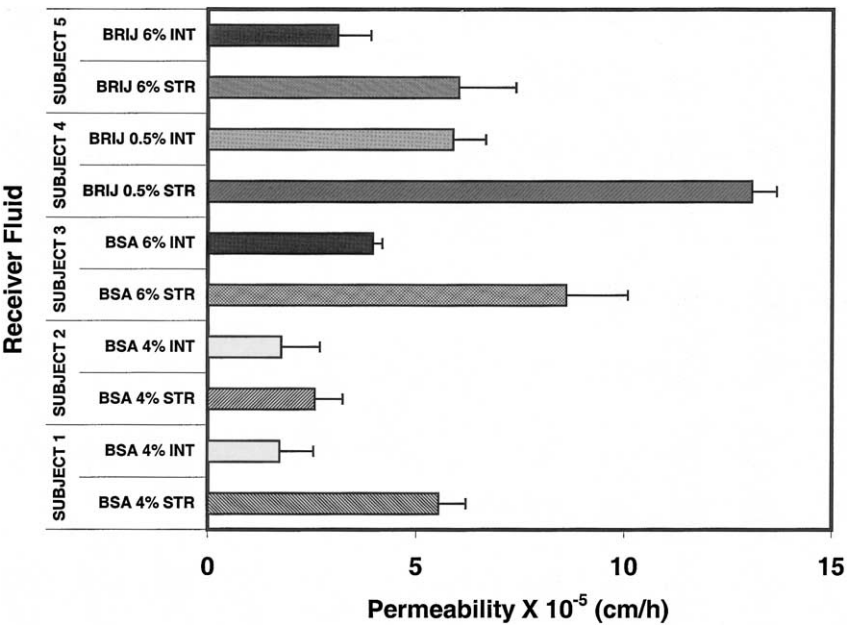


Fig. 6. Human skin permeability of Δ^9-THC: stripped (STR) vs. intact (INT) skin in five subjects. Values are the means with S.D.; $n \geq 3$.

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