In Vitro/*In Vivo* Correlation Studies for Transdermal Δ^8-THC Development

SATYANARAYANA VALIVETI, DANA C. HAMMELL, D. CAROLINE EARLES, AUDRA L. STINCHCOMB

Division of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, Kentucky 40536-0082

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ABSTRACT: The present study was carried out in order to develop a transdermal therapeutic system (TTS) for Δ^8-THC. The *in vitro* permeability studies of Δ^8-THC in human skin and hairless guinea pig skin with and without a rate-controlling membrane were conducted in flow-through diffusion cells. Δ^8-THC pharmacokinetic parameters were determined after topical application of transdermal patches and intravenous administration in guinea pigs. The *in vitro* results indicated that there was no significant difference in the mean flux or in the permeability coefficient of Δ^8-THC in human skin versus hairless guinea pig skin. The flux of Δ^8-THC through the human skin/membrane composite was not significantly lower than that through the hairless guinea pig skin/membrane composite; and the skin controlled the Δ^8-THC delivery rate. Intravenous doses of Δ^8-THC followed a two-compartment model with a significant distribution phase. On application of the TTS patch, the plasma concentration of Δ^8-THC reached a mean steady-state level of 4.4 ng/mL within 1.4 h and was maintained for at least 48 h. Significant amounts of metabolites were observed in the plasma after topical application. The *in vitro*-study predicted plasma concentration following application of the transdermal patch was in agreement with the observed guinea pig plasma concentrations of Δ^8-THC. J Pharm Sci 93:1154–1164, 2004

Keywords: Δ^8-THC; transdermal delivery; transdermal therapeutic system; *in vitro* studies; *in vivo* studies

INTRODUCTION

Cannabinoids are gaining importance for their clinical use in the treatment of nausea and vomiting, appetite loss, pain, muscle spasticity, and movement disorders.[1–3] Δ^9-THC (tetrahydrocannabinol), the main active constituent of the marijuana plant, is marketed under the generic name dronabinol in a capsule form for the treatment of nausea and vomiting caused by anti-neoplastic drugs, and for appetite loss and cachexia in HIV/AIDS patients.[4] The side effects associated with this drug include drowsiness, dizziness in elderly patients, and mood changes in young patients. Δ^8-THC is an isomer of Δ^9-THC and has been shown to be less psychotropic than Δ^9-THC.[5,6] It has been reported that Δ^8-THC prevented vomiting completely when administered before anti-neoplastic therapy in cancer patients, and caused negligible side effects.[6] Cannabinoids are extensively metabolized upon oral administration, which results in low and variable oral bioavailability. Oral doses of cannabinoids can also produce dose-related side effects from high peak drug levels. Moreover, the oral route of drug delivery is not preferable for some severely nauseated patients. Hence, there is a need for developing an alternate dosage form for this class of drugs. Transdermal delivery could be an alternative route for optimization of

Correspondence to: Audra L. Stinchcomb (Telephone: 859-323-6192; Fax: 859-257-2787; E-mail: astin2@email.uky.edu)

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cannabinoid therapy due to its advantages of reducing drug peak-related side effects via zero-order delivery, and eliminating the loss of drug due to first pass hepatic metabolism. Transdermal delivery of cannabinoids could also improve patient compliance in severely nauseated patients.

Very few reports can be found in the scientific literature on the transdermal delivery of cannabinoids.[7–10] There have been no published reports on the development of TTS patches for Δ^8-THC or the other cannabinoids, however, Touitou et al. did the earliest rat (*in vitro* and *in vivo*), mice, and human (*in vitro* only) skin permeation studies with Δ^8-THC from 1988 to 1991.[7–9] The idea of transdermal cannabinoid delivery seems to be very desirable, as first discussed by Touitou et al. in 1988,[7] but the current scientific literature and patent data bases do not contain complete topical dosing pharmacokinetic information from animal or clinical trials. It is necessary to obtain intravenous dose pharmacokinetic parameters, i.e., drug clearance, in order to estimate the *in vivo* transdermal flux for a proper *in vitro*/*in vivo* correlation determination. Additionally, the literature is lacking information on a developed cannabinoid TTS that provides constant steady-state drug levels, as described here. In order to test the permeation characteristics of Δ^8-THC *in vivo* in the best small animal model match for human skin transdermal studies, a TTS was developed. A selective and sensitive liquid chromatography–mass spectrometry (LC–MS) assay and plasma extraction procedure were developed in order to accurately measure levels of Δ^8-THC and its carboxylic acid metabolite. The aim of our Δ^8-THC investigation was subdivided into three goals. Objective one was to compare the *in vitro* permeability of Δ^8-THC in guinea pig and human skin so that any permeation differences could be accounted for when calculating what may happen in humans *in vivo*. Often animal skin is found to be several-fold more permeable than human skin, so a factor can be determined and incorporated into a predictive model for human *in vivo* absorption. The skin permeability of Δ^8-THC could be affected by the TTS rate controlling membrane coated with pressure sensitive adhesive (PSA). Thus, the second objective was to investigate the influence of the rate controlling membrane/PSA on the *in vitro* permeation of the drug. Occasionally, *in vitro* studies can provide an underestimate of the skin flux of very hydrophobic compounds, like the cannabinoids. Therefore, to substantiate our *in vitro* investigation, the third objective was to conduct the *in vivo* permeability studies in the hairless guinea pigs; and to evaluate the ability of the developed TTS to provide a steady-state concentration of the drug. Quantitation of a drug reservoir effect in the skin after patch removal was also an important objective in the *in vivo* studies. The *in vitro*/*in vivo* correlation generated for this drug in the guinea pig model was then used to predict what may happen in humans *in vivo*.

MATERIALS AND METHODS

Chemicals and Films

Δ^8-THC and 11-nor-Δ^8-THC-9-carboxylic acid (THC-COOH) in 95% ethyl alcohol were obtained from Sigma Chemical (St. Louis, MO). Hank's balanced salts modified powder, bovine serum albumin (BSA) fraction V, potassium phosphate monobasic anhydrous, and sodium bicarbonate were also obtained from Sigma Chemical. Propylene glycol, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), triethylamine (TEA), gentamicin sulfate, ammonium acetate, ethyl acetate, and acetonitrile (HPLC grade) were obtained from Fisher Scientific (Fairlawn, NJ). Ethyl alcohol, absolute (200 proof) was obtained from Aldrich Chemical Company (Milwaukee, WI). Water was purified by Millipore Elix 5 reverse osmosis and a Milli-Q® (Millipore, Bedford, MA) Gradient A10 polishing system (Millipore). ARcare®7396 (pressure-sensitive tape with MA-38 medical grade acrylic adhesive and 60# Kraft release paper) was a gift from Adhesives Research, Inc. (Glen Rock, PA). MEDIFLEX® 1502 (backing membrane; pigmented metalized polyester) was a gift from Mylan Technologies, Inc. (St. Albans, VT). ScotchPAK™ 9742, a fluoropolymer release liner, and CoTran™ 9715, a 3 mil ethylene vinyl acetate (EVA) rate controlling membrane with 19% vinyl acetate, were gifts from 3M™ Drug Delivery Systems (St. Paul, MN).

Instruments

Equipment used consisted of PermeGear® flow through diffusion cells of area 0.95 cm^2 with heating blocks (PermeGear, Riegelsville, PA), a Retriever IV Fraction collector (ISCO, Inc., Lincoln, NE), a Pumppro® MPL Static pump (Watson Marlow, Wilmington, MA), a Padgett Dermatome (Padgett Instruments, Kansas City, MO), and a high-pressure liquid chromatography (HPLC) instrument with a 200 series autosampler and a

variable wavelength UV detector model 785A (Perkin Elmer, East Norwalk, CT). The HPLC with MS detection (LC–MS) consisted of a Waters Alliance 2690 HPLC pump (Waters, Milford, MA), a Waters Alliance 2690 autosampler, and a Micromass ZQ detector (Waters).

HHBSS with BSA Receiver Solution

HEPES buffered-Hank's balanced salt solution (HHBSS) was prepared and filtered. Gentamicin sulfate (50 μg/mL) was dissolved in the receiver solution to minimize microbial contamination. BSA (4%) was added to the HHBSS solution. All glassware was rinsed with 70% v/v ethanol prior to HHBSS preparation.

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 samples were either used immediately or frozen at −20°C.

Guinea Pig Skin Preparation

Hairless guinea pigs were sacrificed by pentobarbital overdose. The full thickness skin was removed by blunt dissection and was dermatomed to a thickness of approximately 200 μm. The samples were either used immediately or frozen at −20°C. All animal studies were approved by the University of Kentucky IACUC.

Δ^8-THC Formulation

The Δ^8-THC formulation consisted of a 16 mg/mL drug solution in propylene glycol:water:ethanol (1:1:1).

In Vitro Permeability Studies Across Human Skin or Guinea Pig Skin

The skin surface 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 mounting the human skin or guinea pig skin samples into the cell. The diffusion experiment was initiated by charging the donor compartment with 0.25 mL of drug solution. Each donor cell was capped for the duration of the experiment in order to prevent any formulation evaporation. The HHBSS/BSA receiver solution was pumped through the diffusion cells at a flow rate of 1.1 mL/h for 48 h in order to maintain sink conditions. The solubility of Δ^8-THC in 4% BSA is 28.5 ± 0.9 μg/mL, and the highest concentration of Δ^8-THC in any diffusion sample was 0.77 μg/mL (37-fold less than the solubility in the receiver solution). Receiver solution samples were collected with a fraction collector in 6 h intervals. The diffusion samples were refrigerated until analysis. At the end of the diffusion experiment, the exposed skin area was excised from the skin sample in order to measure tissue drug concentrations. The formulation was rinsed off of the skin with water, tape-stripped two times to remove any residual formulation, and then the weighed and minced tissue was placed in acetonitrile (ACN) to shake at room temperature overnight. The tissue-extracted drug was quantitated by HPLC analysis of the acetonitrile supernatant.

Permeability Studies Across Skin/Rate Controlling Membrane Composite

The EVA rate controlling membrane with the acrylic pressure-sensitive adhesive was mounted on the skin (human skin or guinea pig skin) and the permeation of Δ^8-THC through the skin/membrane composite was also determined. The experimental conditions were the same as those outlined above, except that the membrane composite was placed between the skin and the drug solution.

Sample Preparation

For drug extraction from the BSA diffusion samples, a four-fold volume of ACN was added to each sample in a siliconized microcentrifuge tube. The sample was vortexed for 1 min, sonicated for 15 min, and vortexed for an additional 1 min followed by centrifugation at 10000*g* for 20 min. The supernatant was transferred to silanized autosampler vials and 100 μL of each sample was injected onto the HPLC column. The recovery of Δ^8-THC was found to be $97 \pm 9\%$ when compared to that of drug samples in ACN.

HPLC Analysis of Δ^8-THC

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_{18} Column (Brownlee®, 220 × 4.6 mm, Spheri-5) with a guard column (Brownlee, Reversed phase, C_{18}, 15 × 3.2 mm, 7 μm particle size) was used in the assay. The assay run time was 10 min. The UV detector was set at a wavelength of 215 nm. The retention time for Δ^8-THC was 5.1 ± 0.2 min. Standard curves were linear within the range of 25–1000 ng/mL and the sensitivity of the assay was 25 ng/mL.

In Vivo Studies in Guinea Pigs

Preparation of IV Dosing Solution

Δ^8-THC (1 mg/mL) was prepared in a vehicle of sterile saline with 3%Tween-80:propylene glycol (19:1, v/v). An aliquot of stock of Δ^8-THC (100 mg/mL in ethanol) was evaporated under a stream of nitrogen. Propylene glycol was added and the solution was vortexed, followed by the addition of sterile saline containing Tween-80. The solution was vortexed and sonicated for 5 min. Drug solutions were prepared immediately before each animal was dosed.

Fabrication of Transdermal Patches for Δ^8-THC

The membrane-controlled transdermal therapeutic system (TTS) of Δ^8-THC (7.25 cm^2) was fabricated by sandwiching a drug reservoir between a drug-impermeable backing laminate (MEDIFLEX 1502) and a rate-controlling EVA membrane (CoTran 9715) with ARcare7396 adhesive. A release slip composed of SCOTCHPAK™ 9742 was used to leave a small opening into the reservoir of the empty device. The membrane/adhesive laminate was heat sealed to the metalized polyester backing membrane. The slip was removed to form a small port, and the drug solution (500 μL) of 16 mg/mL Δ^8-THC in 1:1:1(v/v/v) of propylene glycol:water:ethanol was injected into the reservoir. After injecting the drug solution into the reservoir, the port was heat sealed. The TTS patch was kept in a sealed aluminum pouch to minimize the loss of solvent.

Animal Studies

Male and female Hairless IAF and Hartley guinea pigs (Charles River) weighing 359–450 g were used for these studies. Catheters were surgically implanted into the jugular vein. A baseline "blank" plasma sample was drawn from each animal immediately before drug treatment. For IV bolus experiments a dose of 1 mg/kg (1 mL/kg) was infused over a period of 30 s. For topical delivery studies, the developed membrane controlled TTS patches (two patches) were applied to the dorsal region of the hairless guinea pigs. The plasma samples were obtained for 48 h while the patch was on the animal, and another 48 h after patch removal. Plasma samples were drawn for 6 h following the intravenous doses. All animal studies were approved by the University of Kentucky IACUC. The blood samples were immediately centrifuged at 10000*g* for 3 min; plasma was separated and stored at −70°C until analysis by LC-MS.

Plasma Sample Extraction Procedure

Exactly 500 μL of acetonitrile:ethyl acetate (1:1, v/v) was added to 50 μL of plasma sample in a 1.5-mL siliconized microcentrifuge tube, and the mixture was vortexed for 30 s and centrifuged at 10000*g* for 20 min. The supernatant was decanted into a clean silanized test tube, and evaporated under nitrogen at 37°C. The residue was reconstituted with 200 μL of acetonitrile, vortexed, and sonicated for 5 min. The clear solution was placed into a clean HPLC vial containing silanized low volume inserts, and 20 μL of the sample was injected into the LC–MS system. The extraction efficiency was 97 ± 6% for THC and 88 ± 7% for THC-COOH.

LC–MS Analysis of Δ^8-THC and THC-COOH in Plasma Samples

The liquid chromatograph was a Waters Alliance 2690 HPLC pump (Waters) with a Waters Alliance 2690 autosampler and column heater. The analytical column was a Waters Symmetry® C_{18} (2.1 × 150 mm, 5 μm) and the guard column used was a Waters Symmetry® C_{18} (2.1 × 10 mm, 3.5 μm). The mobile phase composition was: (A) 5% 2 mM ammonium acetate in ACN and (B) 2 mM ammonium acetate in water containing 5% ACN. The mobile phase gradient conditions were as follows: 60% A for 5.0 min followed by a linear gradient to 70% A in 1 min, then 70% A for 23 min, and a linear gradient to 60% A in 1 min. Between each run the column was equilibrated for 3 min at 60% A. The flow rate was 0.25 mL/min and the temperature of the column was maintained at 35°C. The volume of injection was 20 μL.

The detector was a Micromass ZQ detector (Waters) equipped with an electrospray ionization (ESI) probe. Selected ion monitoring (SIM) was

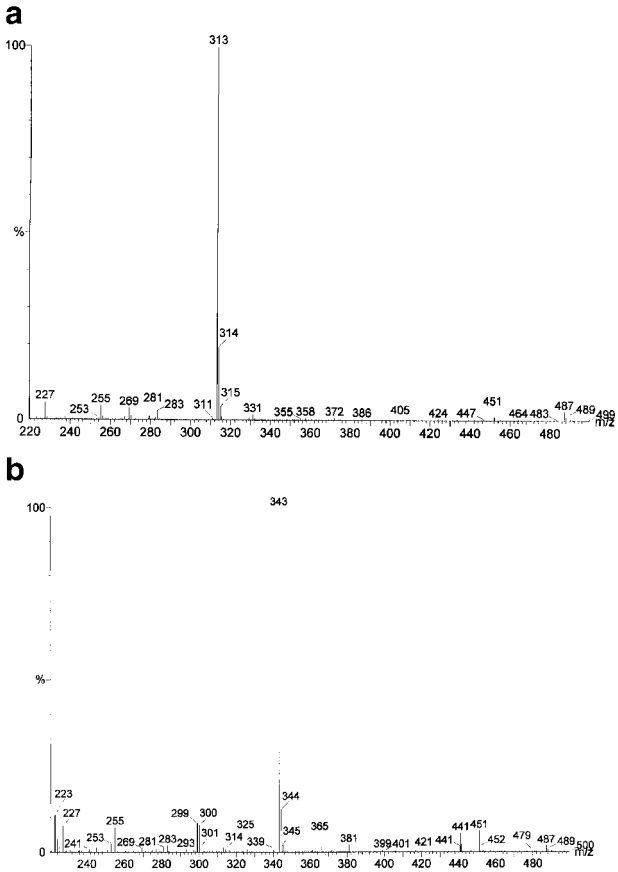

Figure 1. Full scan mass spectrum of (a) Δ^8-THC (m/z 313) and (b) 11-nor-Δ^8-THC-9-COOH (m/z 343).

performed in negative mode for m/z 313 $[THC\text{-}H]^-$ and m/z 343 $[THC\text{-}COOH\text{-}H]^-$ (Fig. 1). The capillary voltage was 4500 V and the cone voltage was 40 V. The source block and desolvation temperatures were 120 and 250°C, respectively. Nitrogen was used as a nebulization and drying gas at flow rates of 50 and 450 L/h, respectively. The required studies were carried out to find the inter- and intra-day variation, and accuracy. The retention times for Δ^8-THC and 11-nor-Δ^8-THC-9-COOH were 27.70–27.95 and 2.98–3.20 min, respectively (Fig. 2). A calibration curve was prepared with each assay at a concentration range of 1.25–200 ng/mL, and the observed correlation coefficient was 0.998 or better. The limit of detection was 1.25 ng/mL for THC and 2.5 ng/mL for THC-COOH.

Data Treatment

In vitro Data Analysis

The diffusion 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^8-THC permeability coefficients were calculated from the steady state flux and the drug concentration in the vehicle. The statistical analysis of data was computed with a one-way ANOVA using SigmaStat (SPSS, Inc., Chicago, IL).

Pharmacokinetic Analysis

The pharmacokinetic analysis of THC plasma concentration versus time profiles after intravenous bolus administration was carried out by fitting the data to a two compartment model (WinNonlin Professional, version 4.0, Pharsight Corporation, Mountain View, CA) with the following exponential expression:

$$C = Ae^{-at} + Be^{-\beta t}. \quad (1)$$

The pharmacokinetic parameters (maximum plasma concentration, C_{max}; elimination rate constant, β; elimination half-life, $t_{1/2(\beta)}$; distribution rate constant, α; distribution half life, $t_{1/2(\alpha)}$; steady state volume of distribution, V_{ss}; area under the

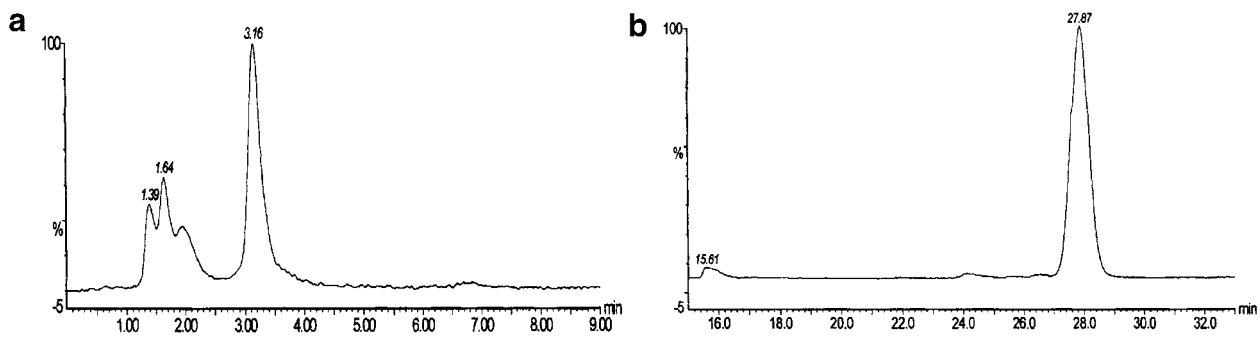

Figure 2. A typical HPLC/MS ion chromatogram for THC and its metabolite in guinea pig plasma (a) 11-nor-Δ^8-THC-9-COOH at 3.16 min and (b) Δ^8-THC at 27.87 min.

curve from 0 to infinity, $AUC_{0-\infty}$; and total body clearance, CL_{tot}) were estimated using the software. The peak plasma concentration (C_{max}) after the IV bolus dose of THC was used to calculated the initial volume of distribution by the following equation:

$$V = \text{Dose}/C_{max}. \quad (2)$$

Following topical administration, data were analyzed by non-compartmental analysis to determine peak concentration (C_{max}), lag time to steady-state concentration (T_{lag}), and area under the curve from 0 to infinity, $AUC_{0-\infty}$.

RESULTS AND DISCUSSION

In the present study, we investigated the *in vitro* transdermal permeability of Δ^8-THC across human skin and hairless guinea pig skin. Although surface lipids, barrier thickness, and morphological aspects are comparable in hairless guinea pig and human skin, variation in the skin permeability of several drugs has been observed.[11,12] Panchagnula et al.[13] investigated the permeability of two different compounds (polar and non-polar) through the skin from 16 different species, including human skin, and found that the permeability of hairless guinea pig skin was closest to that of human skin. In a previous study, it was reported that the permeability of Δ^8-THC was 13-fold higher in rat skin than in human skin.[8] Hence, the present study was conducted in order to compare the *in vitro* permeability of Δ^8-THC in guinea pig skin and human skin; as well as to compare the skin/rate controlling membrane composite influence on the Δ^8-THC permeability. The *in vivo* studies in guinea pigs were conducted in order to examine the ability of the developed TTS of Δ^8-THC to provide a steady-state plasma concentration of the drug; as well as for determination of an *in vitro*/*in vivo* correlation.

In Vitro Permeability Studies

The HPLC method used for quantitative determination of Δ^8-THC in the skin permeation samples was found to be precise and accurate as indicated by a less than 5% coefficient of variation (CV, inter- and intra-day variation) with high recovery. The permeation profiles of Δ^8-THC across human skin and guinea pig skin are shown in Figure 3. The cumulative amount of Δ^8-THC

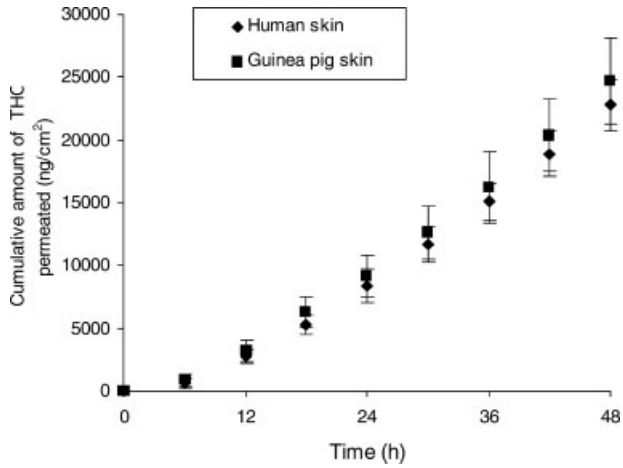

Figure 3. Profile of mean (±SD) cumulative amount of Δ^8-THC permeated through the human skin and guinea pig skin (n = 4).

permeated through the human skin was 22.8 ± 2.0 μg/cm^2 and was not significantly lower ($p > 0.05$) than that through guinea pig skin (24.7 ± 3.4 μg/cm^2). The *in vitro* permeation parameters of Δ^8-THC through human skin and guinea pig skin are given in Table 1. The calculated steady state flux was 649 ± 64 ng/cm^2/h for human skin and 717 ± 59 ng/cm^2/h for guinea pig skin with corresponding lag times of 12.4 ± 2.1 and 13.2 ± 2.8 h, respectively. There was no significant ($p > 0.05$) difference observed in the flux, lag times, nor drug content in the skin of the guinea pig compared with human skin. No metabolite (11-nor-Δ^8-THC-9-COOH) of Δ^8-THC was found in the *in vitro* skin permeability samples. The results of the *in vitro* data indicated that the permeability of Δ^8-THC in guinea pig skin was slightly higher than that in human skin, but the difference was not statistically significant ($p > 0.05$). It appears from the *in vitro* data that guinea pig skin is a good model for human Δ^8-THC permeability studies. The most significant barrier to diffusion of highly hydrophobic drugs like THC is usually the viable tissue, rather than the stratum corneum.[10] It makes sense that guinea pig skin provides a very accurate estimate for human skin permeation of a drug that is rate-limited by the viable epidermis, rather than rate-limited by the stratum corneum. The diversity of the stratum corneum structure and function among species is far more likely to provide permeation differences than the sub-stratum corneum aqueous viable tissues.

Table 1. *In Vitro* Permeation Parameters for Δ^8-THC in Human Skin and Guinea Pig Skin (n = 4)

Skin	Flux (ng/cm^2/h) Mean ± SD	Permeability $\times 10^5$(cm/h) Mean ± SD	Skin Concentration (μg drug/g Skin) Mean ± SD	Lag Times (h) Mean ± SD
Human skin	649 ± 64	4.05 ± 0.40	4062 ± 1281	12.4 ± 2.1
Guinea pig skin	717 ± 59	4.48 ± 0.37	2673 ± 377	13.2 ± 2.8
Human skin/membrane composite	647 ± 89	4.05 ± 0.56	—	15.1 ± 2.0
Guinea pig skin/membrane composite	709 ± 83	4.43 ± 0.52	—	16.8 ± 1.6

In Vitro Permeability Studies of Δ^8-THC Through Skin/Membrane Composite

The permeation of Δ^8-THC through the skin/membrane composite was studied in order to help develop a TTS, and to provide data for *in vitro*/*in vivo* correlation. Formation of a Δ^8-THC reservoir on the skin surface was accomplished by using an EVA copolymer membrane. Intimate contact of the transdermal patch with the skin was achieved with an acrylic pressure-sensitive adhesive. Since the skin permeability of Δ^8-THC could be affected by the EVA rate controlling membrane and PSA, the composite of the rate controlling membrane coated with the adhesive was studied in the human and guinea pig skin *in vitro*. The cumulative permeation profiles of Δ^8-THC in the human skin/membrane composite and guinea pig skin/membrane composite are shown in Figures 4 and 5, respectively. The cumulative amount of Δ^8-THC permeated across the human skin/membrane composite and guinea pig skin/membrane composite was 21065 ± 2122 and 21824 ± 2167 ng/cm^2, respectively. The permeation parameters of Δ^8-THC through the human skin/membrane composite and guinea pig skin/membrane composite are given in Table 1. These permeability parameters of Δ^8-THC in human skin/membrane composite were not significantly different ($p > 0.05$) from those in guinea pig skin/membrane composite.

The *in vitro* permeation studies with the membrane composites also provided valuable information about the drug delivery rate control. The flux and lag time of Δ^8-THC through the human skin/membrane composite (647 ± 89 ng/cm^2/h, 15.1 ± 2.0 h, respectively) and guinea pig skin/membrane composite (709 ± 83 ng/cm^2/h, 16.8 ± 1.6 h, respectively) were slightly different than that through human skin alone (649 ± 64 ng/cm^2/h, 12.4 ± 2.1 h, respectively) and guinea pig skin alone (717 ± 59 ng/cm^2/h, 13.2 ± 2.8 h, respectively), but not statistically significant ($p > 0.05$). This indicated that the EVA membrane with the

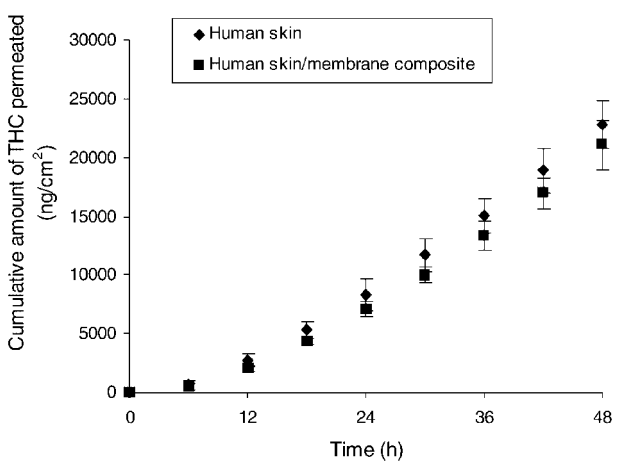

Figure 4. Profile of mean (±SD) cumulative amount of Δ^8-THC permeated through the human skin with or without rate controlling membrane/adhesive layer (n = 4).

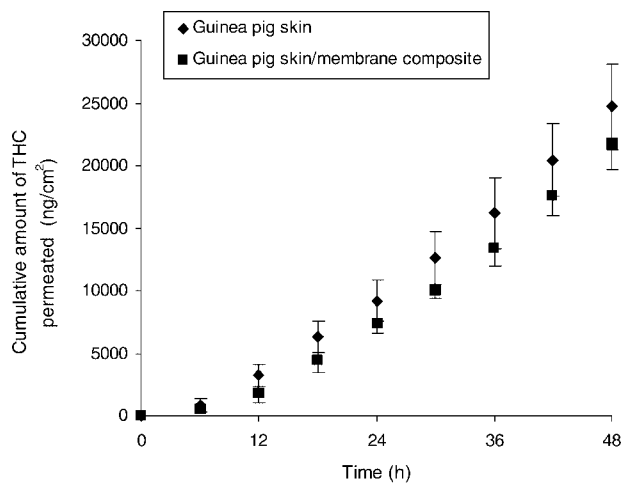

Figure 5. Profile of mean (±SD) cumulative amount of Δ^8-THC permeated across the guinea pig skin with or without rate controlling membrane/adhesive layer (n = 4).

adhesive had no significant influence on the permeability of Δ^8-THC through human or guinea pig skin, and therefore, the skin controlled the delivery rate in both species.

In Vivo Studies in Guinea Pigs

Intravenous Administration of Δ^8-THC

Prediction of the *in vivo* plasma levels of Δ^8-THC in the guinea pig from the *in vitro* permeability data required determination of pharmacokinetic parameters, including drug clearance. There is no pharmacokinetic data available for Δ^8-THC in the guinea pig in literature resources. Hence, the pharmacokinetic parameters were calculated after intravenous administration of Δ^8-THC (1 mg/kg) in guinea pigs. The plasma profile of observed and predicted concentrations after Δ^8-THC intravenous administration is shown in Figure 6. The observed and predicted data were in agreement (correlation = 0.65) with a two compartment open model. The maximum plasma concentration of Δ^8-THC was 642 ± 121 ng/mL. The plasma levels of Δ^8-THC fell rapidly to an average of 89 ng/mL at 60 min and 11 ng/mL at 6 h. The two-compartment model plasma concentration time course has also been observed in humans after Δ^9-THC dosing.[14] The pharmacokinetic parameters of Δ^8-THC after intravenous administration are given in Table 2. After an intravenous bolus dose, the mean half life for the distribution phase ($t_{1/2(\alpha)}$) and the mean elimination half life ($t_{1/2(\beta)}$) were 0.27 and 3.52 h, respectively. The steady state volume of distribution and clearance for Δ^8-THC were 6.6 L/kg and 2.39 L/h, respectively. The volume of distribution was over 100 times higher than guinea pig plasma volume,[15] indicating extensive distribution to the tissues (as seen in humans).[14] The metabolite of Δ^8-THC, 11-nor-Δ^8-THC-9-COOH, was not detected in the guinea pig plasma up through 6 h of sampling.

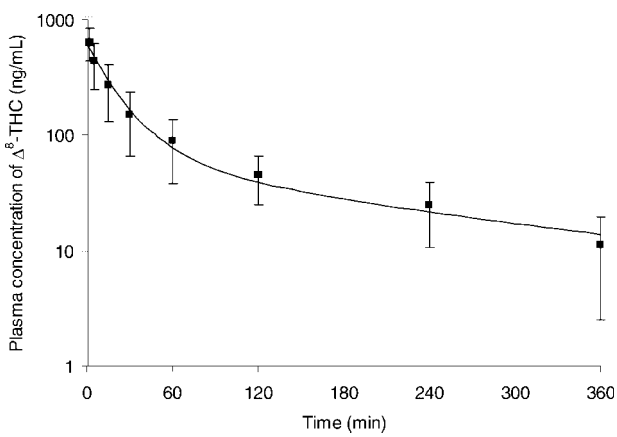

Figure 6. Mean (±SD) plasma profile of Δ^8-THC after intravenous administration (1 mg/kg) in guinea pigs (n = 7).

Table 2. Pharmacokinetic Parameters of Δ^8-THC After Intravenous Administration (1 mg/kg) in Guinea Pigs (n = 7)

Parameter	Mean ± SD
AUC (ng/mL × h)	490 ± 233
α (h^{-1})	3.9 ± 1.6
β (h^{-1})	0.27 ± 0.16
$t_{1/2(\alpha)}$ (h)	0.21 ± 0.09
$t_{1/2(\beta)}$ (h)	3.5 ± 2.0
C_{max} (ng/mL)	642 ± 121
CL (L/h)	2.39 ± 1.34
AUMC (ng/mL × h^2)	1782 ± 1701
MRT (h)	3.2 ± 1.6
V_{ss} (L/kg)	6.6 ± 3.0
Initial V (L/kg)	1.6 ± 0.3
Weight (kg)	0.392 ± 0.031

Application of the Developed TTS Patch of Δ^8-THC

The individual plasma concentration profiles of Δ^8-THC and its metabolite at different time points following the application of the TTS patch are shown in Figure 7a. The plasma concentration of Δ^8-THC gradually increased and attained an average steady state level of 4.4 ± 0.9 ng/mL at about 1.4 ± 0.7 h (lag time). The steady-state levels were maintained for more than the 48 h application period, because the steady-state plasma concentrations were maintained for another 24 h after TTS patch removal, indicating a prolonged skin reservoir effect for the highly lipophilic drug. The steady-state concentration of the drug did decline gradually after 72 h. Thus, the steady state concentration of Δ^8-THC (4.4 ± 0.9 ng/mL) was maintained for about 70 h (without the lag time). The pharmacokinetic parameters of Δ^8-THC, including C_{max}, C_{ss}, $AUC_{0-\infty}$, and T_{lag} (time to reach steady state plasma concentration) following application of the TTS patch are given in Table 3. A mean steady state concentration of 4 ng/mL of 11-nor-Δ^8-THC-9-COOH was attained at about 18.5 h (Fig. 7b).

The predicted steady state plasma concentration of Δ^8-THC in the guinea pig following the

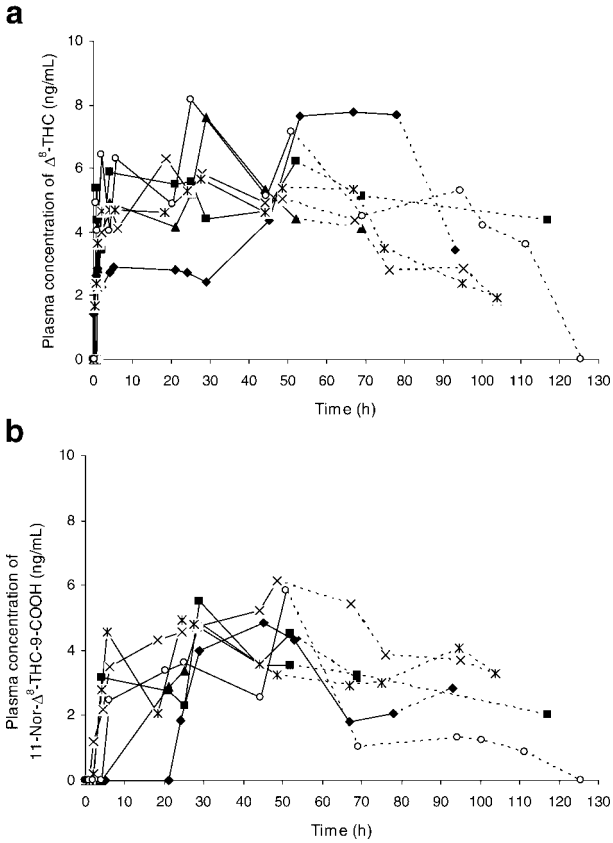

Figure 7. Individual plasma profiles of (a) Δ^8-THC and (b) its metabolite (THC-COOH) after the application of transdermal therapeutic system (TTS) patches in guinea pigs. Dotted lines indicate plasma levels after the patch was removed.

application of the TTS patch can be calculated as 4.3 ng/mL from the *in vitro* steady state flux. This was calculated by using the following equation:

$$C_{ss} = \frac{J_{ss}A}{CL}, \qquad (3)$$

where 'C_{ss}' is the predicted steady plasma concentration (ng/mL); 'J_{ss}' is the steady state flux (709 ng/cm^2/h); 'A' is area of the applied patch (14.5 cm^2); 'CL' is the total body clearance

Table 3. Pharmacokinetic Parameters of Δ^8-THC After the Application of the TTS Patch in Hairless Guinea Pigs (n = 6)

Parameter	Mean ± SD
AUC (ng/mL × h)	766 ± 354
C_{max} (ng/mL)	6.6 ± 1.5
T_{lag} (h)	1.4 ± 0.7
C_{ss} (ng/mL)	4.4 ± 0.9

(2390 mL/h). In the present study, the mean steady-state plasma concentration following the application of the TTS patches in the guinea pigs was 4.4 ng/mL for Δ^8-THC. The observed steady state plasma concentration of Δ^8-THC was in very good agreement with the predicted plasma concentration of Δ^8-THC (4.3 ng/mL) obtained from the *in vitro* data. This excellent *in vitro*/*in vivo* correlation tells us that further formulation studies for the final prototype patch can be developed with the *in vitro* experiments.

Prediction of Patch Performance in Humans

Equation 3 can be used to predict the steady-state plasma concentration that would be obtained in humans. The human skin flux value, 647 ng/cm^2/h, obtained in the *in vitro* experiments should provide a good estimate of *in vivo* flux, based on the excellent guinea pig *in vitro*/*in vivo* correlation obtained here. Specific Δ^8-THC human clearance data is not available in the literature, therefore the Δ^9-THC human clearance value (13 L/h) can be used to estimate Δ^8-THC's value.[16] This seems to be a reasonable estimate, as both isomers have similar physicochemical properties and are biotransformed by identical oxidative mechanisms.[17] If a 10–20 ng/mL plasma concentration is targeted, assuming that Δ^8-THC is similar in potency to half as potent as Δ^9-THC,[6] then further formulation optimization (perhaps including permeation enhancers) would need to be done in order to achieve a four to eight-fold increase in flux. This would be necessary so that a reasonable patch size limitation of 50 cm^2 could be used. This should be easy to achieve with simple formulation manipulation using the validated *in vitro* and *in vivo* models. Many strategies to improve the permeation rates of hydrophobic drugs have been examined by other laboratories. Successful strategies for flux enhancement include microemulsion systems with enhancers,[18] cyclodextrin complexation,[19] supersaturation,[20] and the addition of penetration enhancer combinations.[21] The enhancer *n*-methyl pyrrolidone improved the partitioning and concentration of the hydrophobic drug estradiol in an oil/water microemulsion so that the permeation enhancement was greater than 58-fold that from an isopropyl myristate formulation.[18] Cyclodextrin complexation and supersaturation also increase the concentration (solubility) and stratum corneum partition coefficients of hydrophobic compounds,[19,20] whereas it is also possible to increase

the diffusivity of hydrophobic compounds by using a penetration enhancer combination like propylene glycol and lauric acid.[21]

CONCLUSIONS

The present study was carried out to develop a membrane controlled TTS for Δ^8-THC. The *in vitro* transdermal flux of Δ^8-THC through human skin and guinea pig skin was found to be 649 ± 64 and 717 ± 59 ng/cm^2/h with a lag period of 12.4 and 13.2 h, respectively. There was no significant difference ($p > 0.05$) in flux nor lag time in human skin and guinea pig skin. This indicated that guinea pig skin could be an alternative to human skin *in vitro* studies with Δ^8-THC, however, future penetration enhancer studies might not show the same consistent results. *In vitro* permeation across skin/membrane composites was studied in order to investigate the influence of a rate controlling membrane with an adhesive layer on the permeability of Δ^8-THC across the skin. The results indicated that this membrane had no significant ($p > 0.05$) influence on the permeability of Δ^8-THC. *In vivo* pharmacokinetic studies were conducted by intravenous administration (1 mg/kg) of Δ^8-THC and application of a developed TTS patch in guinea pigs. The results of the *in vivo* studies indicated that a mean steady-state concentration of 4.4 ng/mL was maintained for at least 48 h. A significant amount of metabolite, 11-nor-Δ^8-THC-9-COOH (mean steady-state concentration of 4.0 ng/mL) was observed after application of the TTS patch in guinea pigs. This metabolite is known to have some pharmacologic activity, and would contribute to the overall effectiveness of the eventual transdermal patch. The observed *in vivo* results from the TTS in the guinea pig matched the prediction from the *in vitro* data very well, and this correlation will be invaluable in further TTS formulation optimization and prototype patch development for this highly lipophilic drug.

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