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Human skin permeation of Δ^8-tetrahydrocannabinol, cannabidiol and cannabinol

Audra L. Stinchcomb, Satyanarayana Valiveti, Dana C. Hammell and Diana R. Ramsey

Abstract

The purpose of this study was to quantify the in-vitro human skin transdermal flux of Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabidiol (CBD) and cannabinol (CBN). These cannabinoids are of interest because they are likely candidates for transdermal combination therapy. Differential thermal analysis and in-vitro diffusion studies with human tissue were completed for the compounds. Heats of fusion, melting points and relative thermodynamic activities were determined for the crystalline compounds, CBD and CBN. Flux, permeability, tissue concentration and lag times were measured in the diffusion experiments. CBN had a lower heat of fusion and corresponding higher calculated relative thermodynamic activity than CBD. Ethanol concentrations of 30 to 33% significantly increased the transdermal flux of Δ^8-THC and CBD. Tissue concentrations of Δ^8-THC were significantly higher than for CBN. Lag times for CBD were significantly smaller than for CBN. The permeabilities of CBD and CBN were 10-fold higher than for Δ^8-THC. Combinations of these cannabinoids with ethanol will be further studied in transdermal patch formulations in vitro and in vivo, as significant flux levels of all the drugs were obtained. CBD, the most polar of the three drugs, and other more polar cannabinoids will also be the focus of future drug design studies for improved transdermal delivery rates.

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

Audra L. Stinchcomb, Satyanarayana Valiveti, Dana C. Hammell, Diana R. Ramsey

Correspondence:
Audra L. Stinchcomb, Division of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082, USA.
E-mail: astin2@email.uky.edu

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Introduction

Cannabinoid derivatives are gaining recognition in ongoing clinical trials for treatments such as appetite stimulation and weight gain in AIDS and cancer patients, nausea and vomiting side-effect relief associated with cancer chemotherapy, and as a unique addition to pain therapy regimens. Furthermore, a recent clinical trial with an oral cannabis extract and dronabinol (Δ^9-THC) showed evidence of a significant treatment effect in the patient-reported spasticity and pain of multiple sclerosis (Zajicek et al 2003). Zero-order transdermal drug delivery of cannabinoids may help to reduce the bothersome peak drug level-related side-effects seen with oral and inhalation dosing (Vinciguerra et al 1988). Transdermal delivery would also bypass the first-pass metabolism of those cannabinoids with poor oral bioavailabilities. Currently, a patient taking dronabinol capsules as an antiemetic must take one dose 1 to 3 h before chemotherapy, and then every 2 to 4 h after chemotherapy is complete for a total of four to six doses (Weiner 1996). This dosing regimen is quite aggressive, especially in a patient who is not feeling generally well. Swallowing all those capsules when nauseated is not extremely practical either. Applying a transdermal patch several hours before chemotherapy, and then leaving it on for a day or two during the most likely nausea and vomiting period would be more convenient. Additionally, wearing a patch for a week to stimulate appetite would be superior to twice a day oral dosing with dronabinol. The drug abuse potential of the cannabinoid transdermal patches would be small because the plasma drug levels would rise slowly after patch application, causing any psychotropic drug effects to be curbed, as well as delayed for many hours (Grotenhermen 2003).

Evidence continues to surface supporting the fact that combinations of cannabinoids may provide benefits that surpass treatment with single cannabinoids (Grinspoon &

Bakalar 1997). Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabinol (CBN) and cannabidiol (CBD) occur naturally in the plant *Cannabis sativa*. Ideally, a drug co-administration interaction will result in heightened pharmacodynamic therapeutic response without significant changes in drug metabolism and other pharmacokinetic parameters. In the real world, increased drug levels can sometimes mean increased side-effects, so pharmacodynamic changes that decrease side-effects or improve therapeutic effect are desirable. CBD has been reported to decrease some of the side-effects of Δ^9-THC (Dalton et al 1976). Most of the cannabinoid combination therapy data points to the promise of co-administration of THC and CBD (Karniol et al 1974; Hollister & Gillespie 1975; Dalton et al 1976; Zuardi et al 1982; Bornheim et al 1995; Jaeger et al 1996; Petitet et al 1998; McArdle et al 2001). One clinical report describes increased effects with Δ^9-THC and CBN (Karniol et al 1975). CBD is gaining more popularity in clinical cannabis extract trials in Europe, and it seems the drug may be a useful therapy with and without a Δ^9-THC combination (GW Pharmaceuticals). Other successful CBD clinical studies have previously been completed for treatment of psychosis, dystonic movement disorders and epilepsy (Cunha et al 1980; Consroe et al 1986; Sandyk et al 1986; Zuardi et al 1995).

Δ^9-THC, Δ^8-THC and CBN are tetrahydropyran analogues and CBD is an open pyran ring analogue (Mechoulam & Shvo 1963), as shown in Figure 1. Only four reports on the transdermal delivery of cannabinoids exist in the literature. Three reports are from the same author, and the fourth is a Δ^9-THC study published by this laboratory in 2002 (Challapalli & Stinchcomb 2002). Two of the studies describe the successful delivery of Δ^8-THC across rat and human skin with the help of oleic acid and transcutol penetration enhancers (Touitou et al 1988; Fabin & Touitou 1991). The third study describes enhanced transdermal delivery of Δ^8-THC using decylmethylsulfoxide and oleic acid on hairless mouse skin (Touitou & Fabin 1988). Δ^8-THC is slightly less potent than Δ^9-THC, and is of interest for drug delivery because it is more stable and has a similar pharmacologic profile to Δ^9-THC.

The focus of the present study is on the in-vitro human skin transdermal flux of three cannabinoids that are likely candidates for combination patch therapy, Δ^8-THC, CBN and CBD.

Materials and Methods

Chemicals

Δ^8-THC in 95% ethyl alcohol was obtained from Sigma Chemicals (St Louis, MO). CBN and CBD were obtained from the National Institute on Drug Abuse (Research Triangle Park, NC). Hank's balanced salts modified powder, bovine serum albumin fraction V (BSA), potassium phosphate monobasic anhydrous, sodium bicarbonate and polyoxyethylene 20 oleyl ether (Brij 98) were obtained from Sigma Chemicals (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).

Instruments

The 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 an HPLC instrument with a 200 series autosampler and a variable wavelength UV detector model 785A (Perkin Elmer, CT). The differential scanning calorimeter was model DSC 2920 (TA Instruments Inc., New Castle, DE).

In-vitro diffusion studies

Receiver solutions

HHBSS with BSA HEPES buffered-Hank's balanced salt solution (HHBSS) was prepared and filtered. Gentamicin sulfate (50 $\mu g\,mL^{-1}$) was dissolved in the recei-

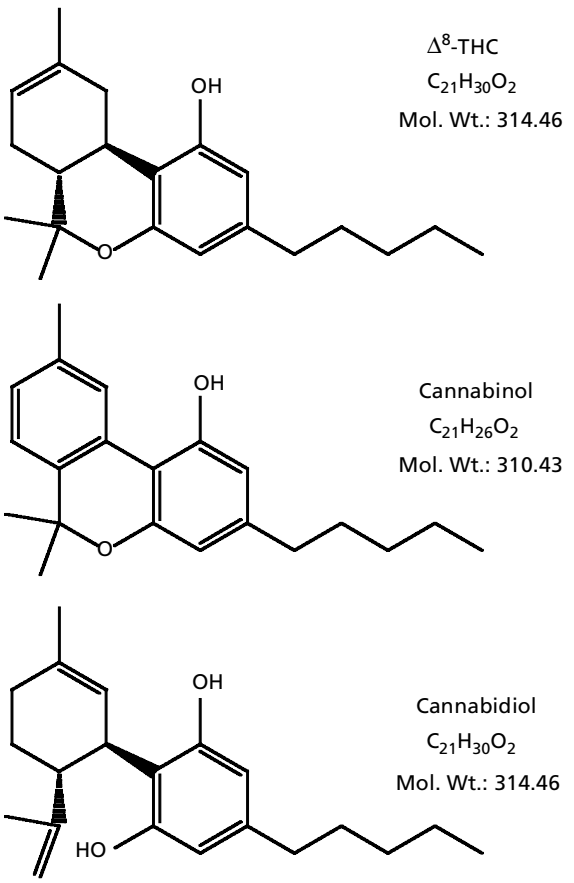

Figure 1 Chemical structures of Δ^8-THC, CBN and CBD.

ver solution to minimize microbial contamination. 4% BSA was added to the HHBSS solution. All glassware used was rinsed with 70% v/v ethanol prior to HHBSS preparation.

Brij 98 solution Five grams of Brij 98 (for 0.5% w/v) was weighed out and dissolved in 1000 mL of filtered distilled water to make the receiver solution. 6% Brij 98 was also used in a few CBD and Δ^8-THC experiments.

Human skin preparation
Human skin samples from abdominoplasty surgery were obtained from the National Cancer Institute's Cooperative Human Tissue Network. The samples were dermatomed immediately on arrival to a thickness of approximately 200 μm. The samples were either used immediately or frozen at –20 °C. University of Kentucky Institutional Review Board approval was obtained for the use of this human tissue.

Δ^8-THC, CBD and CBN formulations
The Δ^8-THC formulation consisted of a 9.09 mg mL^{-1} drug solution in propylene glycol:water:ethanol (9:1:1) or 16.67 mg mL^{-1} in propylene glycol:water:ethanol (1:1:1). The CBD and CBN formulations consisted of saturated solutions (containing excess solid) in mineral oil, 7:3 propylene glycol:water or 4:5:4 propylene glycol:water:ethanol. Solubility studies were completed for CBD and CBN in 7:3 propylene glycol:water and 4:5:4 propylene glycol: water:ethanol. An excess quantity of CBD or CBN was added to a siliconized microcentrifuge tube containing 500 μL of 7:3 propylene glycol:water or 4:5:4 propylene glycol:water:ethanol, and allowed to equilibrate in a shaking incubation oven set to 32 °C (skin surface temperature) for 48 h. A 50 μL sample of the centrifuged supernatant was diluted with 950 μL of acetonitrile, then 50 μL of this solution was further diluted with 950 μL acetonitrile, and this was analysed by HPLC.

In-vitro experimental conditions
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 securing the 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 receiver solution was pumped through the diffusion cells at a flow rate of 1.1 mL h^{-1} for 48 h. Receiver solution samples were collected with a fraction collector at 6 h intervals. The diffusion samples were refrigerated until analysis. At the end of the diffusion experiment, the treated skin area was excised from the skin sample in order to measure tissue drug concentrations. The formulation was rinsed off the skin with water, tape-stripped twice to remove any residual formulation, and then the weighed and minced tissue was placed in acetonitrile to shake at room temperature overnight. The tissue-extracted drug was quantified by HPLC analysis of the acetonitrile supernatant.

Sample preparation

BSA samples
For drug extraction from the BSA diffusion samples, a 4-fold volume of acetonitrile was added to each sample in a siliconized microcentrifuge tube. The sample was vortexed for 1 min, sonicated for 15 min, then vortexed for an additional 1 min followed by centrifugation at 10 000 $\times$ *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 recoveries of Δ^8-THC, CBD and CBN from BSA solutions were found to be 97 $\pm$ 9%, 98 $\pm$ 8% and 94 $\pm$ 5%, respectively.

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

HPLC assay (Δ^8-THC, CBD and CBN)

The mobile phase consisted of 80:20 acetonitrile:phosphate buffer (25 mM KH_2PO_4 + 0.1% TEA, pH 3.0) set at a flow rate of 1.5 mL min^{-1}. A reversed-phase C_8 column (Brownlee, 220 $\times$ 4.6 mm, Spheri-5) with a guard column (Brownlee, reversed phase, C_8, 15 $\times$ 3.2 mm, 7 μm particle size) was used in the assay. The assay run time was 10 min, except for the Brij 98 samples, which were run for an additional 7 min at a flow rate of 2.0 mL min^{-1} to wash the Brij 98 from the column. The UV detector was set at a wavelength of 215 nm. Standard curves were linear within the range of 25–1000 ng mL^{-1} and the limit of detection was 5 ng mL^{-1}. The retention times for Δ^8-THC, CBD and CBN were 5.1 $\pm$ 0.2 min, 3.5 $\pm$ 0.1 min and 4.2 $\pm$ 0.2 min, respectively.

Data treatment

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.

Statistical analysis

Statistical analysis of three separate determinations of the solubilities of CBD and CBN were performed using a Student's *t*-test (Excel, Microsoft Corp., Redmond, WA). The statistical analysis of the flux, permeability, skin concentration and lag time data was completed with a one-way ANOVA. In all cases, post-hoc comparisons of the means of individual groups were performed using Tukey's honestly significant difference test with SigmaStat (SPSS Inc.,

Chicago, IL). A significance level of $P < 0.05$ denoted significance in all cases.

Differential scanning calorimetry for CBD and CBN

Differential scanning calorimetry (DSC) was carried out for the two crystalline cannabinoids, CBD and CBN. The heats of fusion, ΔH_f, and melting points were determined with a TA Instruments 2920 DSC. An accurately weighed sample of drug (4–6 mg) was placed in the aluminium pan and heating curves were recorded at $10\,^{\circ}C\,min^{-1}$. All measurements were repeated twice for a total of three scans on each drug.

Results and Discussion

The differential thermal analysis data for CBD and CBN is shown in Table 1. Both drugs exhibited only one thermal transition, and the endotherm peaks corresponded to the melting of the crystals. The heats of fusion were calculated from the areas under the melting peak curves. Melting points and heats of fusion are important physicochemical determinants of solubility. This solubility or drug solution saturation condition provides the maximum thermodynamic activity driving force for drug transport across the skin. Therefore, reference state thermodynamic activities were calculated from the equation in Table 1 using room temperature (25 °C or 298 K) (Hildebrand & Scott 1950; Stinchcomb et al 1995). A significantly smaller heat of fusion and corresponding higher relative thermodynamic activity were observed for CBN, as compared to CBD. One can predict that CBN may have a higher oil solubility and possible increased chance of crossing the intercellular lipid barrier of the stratum corneum at a faster rate than CBD, if their diffusion coefficients are not substantially different and the stratum corneum is the rate-determining barrier to diffusion. The polar solvent (7:3 propylene glycol:water) solubility of CBD was significantly higher than that of CBN, $5.72 \pm 0.19\,\mu mol\,mL^{-1}$ and $5.35 \pm 0.16\,\mu mol\,mL^{-1}$, respectively (Student's t-test, $P < 0.05$). This corresponds with the prediction that CBN has a higher oil/nonpolar solubility than CBD. The presence of the additional hydroxyl group in the CBD structure corresponds with the slightly increased polar solvent solubility, as compared to the CBN structure. The lower heat of fusion and higher relative thermodynamic activity of CBN did not result in a significantly higher flux of CBN across the skin.

Table 1 Differential thermal analysis of CBD and CBN.

	CBD	CBN
Molecular weight ($g\,mol^{-1}$)	314.46	310.43
Melting point, T_f (°C, ±s.d., n = 3)	67.5 ± 0.3	79.0 ± 0.4
Heat of fusion, ΔH_f (kJ mol^{-1}, ± s.d., n = 3)	28.4 ± 2.5	17.0 ± 0.6
Mean activity of solid @ $T = 25\,^{\circ}C$, a_2[a]	0.24	0.35

[a]Calculated from $\ln a_2 = \frac{-\Delta H_f}{RT}\left(\frac{T_f - T}{T_f}\right)$ (from Hildebrand & Scott 1950).

Table 2 shows a summary of all the in-vitro human skin diffusion study data for all three drugs. Individual permeation profiles for Δ^8-THC, CBN and CBD are plotted in Figures 2, 3 and 4, respectively. Ethanol concentrations of 30–33% significantly enhanced the flux of Δ^8-THC ($P < 0.01$, Figure 2) and CBD ($P < 0.001$, Figure 4), and may prove to be a useful addition to eventual cannabinoid transdermal patches. CBD with 30.8% ethanol provided a flux significantly higher than the Δ^8-THC with 33.3% ethanol ($P < 0.05$). However, the ethanol flux enhancement for Δ^8-THC seems to be due to a permeability increase, and the enhancement of CBD seems to be due to a solubility increase ($5.7 \pm 0.2\,\mu mol\,mL^{-1}$ in 7:3 propylene glycol:water vs $16.2 \pm 0.5\,\mu mol\,mL^{-1}$ in 4:5:4 propylene glycol:water:ethanol). The permeabilities of CBD and CBN are 10-fold higher than those of Δ^8-THC and ^{9}-THC (Challapalli & Stinchcomb 2002). Although some stratum corneum resistance was observed in the human skin per-

Table 2 Drug diffusion properties for Δ^8-THC, CBN and CBD.

Drug	Formulation/receiver solution, n = 2–4	Mean flux ($nmol\,cm^{-2}\,h^{-1}$) ± s.d.	Permeability $\times 10^{-5}$ ($cm\,h^{-1}$) ± s.d.	Skin conc. ($\mu mol\,drug\,g^{-1}$ skin) ± s.d.	Lag times (h) ± s.d.
Δ^8-THC	9:1:1[a]/0.5%Brij	1.00 ± 0.01	3.5 ± 0.1	32.4 ± 17.8	16.0 ± 0.8
	9:1:1/4%BSA	0.81 ± 0.07	2.8 ± 0.2	50.6 ± 32.9	15.5 ± 2.6
	1:1:1/6%Brij	3.12 ± 0.51	5.9 ± 1.0	ND[c]	1.2 ± 1.2
CBN	7:3:0/0.5%Brij	2.31 ± 0.25	43 ± 4.7	18.9 ± 3.2	19.5 ± 1.8
	7:3:0/4%BSA	0.81 ± 0.17	15 ± 3.2	13.7 ± 8.7	20.9 ± 4.1
CBD	7:3:0/0.5%Brij	1.66 ± 0.30	29 ± 5.2	4.4 ± 1.8	10.3 ± 2.8
	7:3:0/4%BSA	1.40 ± 1.07	24 ± 1.9	19.4 ± 8.4	14.4 ± 4.0
	4:5:4/6%Brij	4.69 ± 0.73	29 ± 4.5	ND	0
	MO[b]/6%Brij	0.73 ± 0.18	ND	ND	10.5 ± 5.8

[a]Ratio of propylene glycol:water:ethanol. [b]Mineral oil. [c]Not determined.

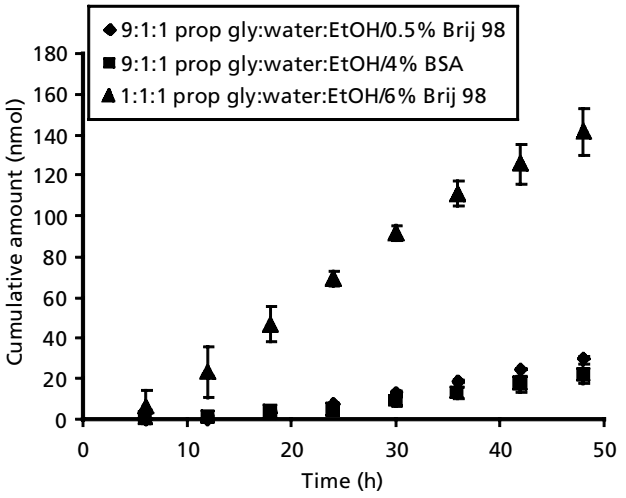

Figure 2 Δ^8-THC permeation profiles: formulation/receiver solutions of 9:1:1 propylene glycol:water:ethanol/0.5% Brij 98 (skin from one subject), 9:1:1 propylene glycol:water:ethanol/4% BSA (skin from three subjects) and 1:1:1 propylene glycol:water:ethanol/6% Brij 98 (skin from one subject). Values are the means with standard deviation; n = 2 to 4 cells per subject's skin sample.

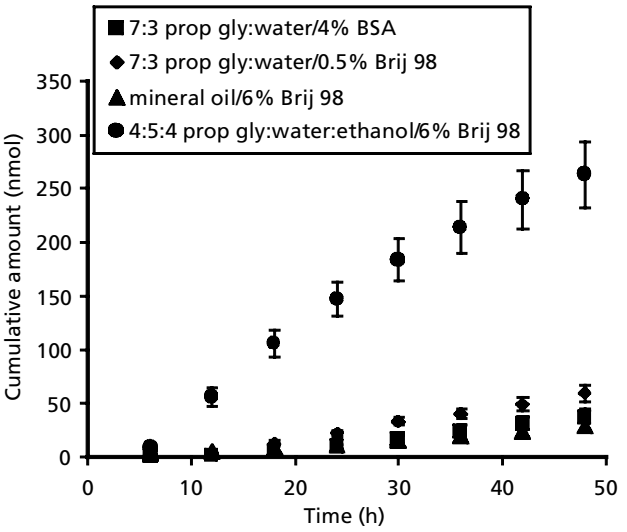

Figure 4 CBD permeation profiles: formulation/receiver solutions of 7:3 propylene glycol:water/0.5% Brij 98 (skin from one subject), 7:3 propylene glycol:water/4% BSA (skin from three subjects), mineral oil/6% Brij 98 (skin from two subjects), 4:5:4 propylene glycol:water: ethanol/6% Brij 98 (skin from one subject). Values are the means with standard deviation; n = 2 to 4 cells per subject's skin sample.

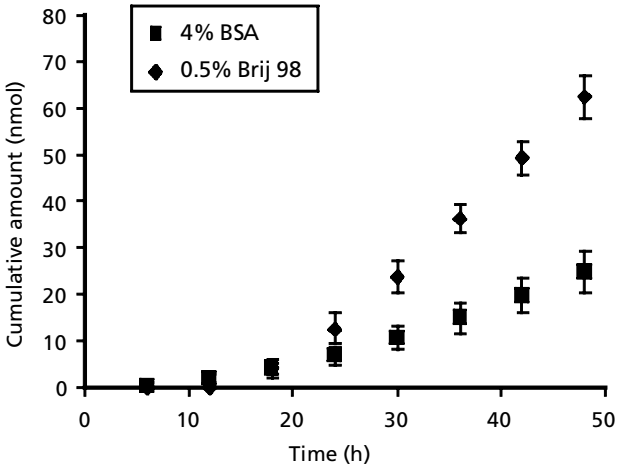

Figure 3 CBN permeation profiles: receiver solutions of 0.5% Brij 98 (skin from one subject) or 4% BSA (skin from five subjects). Values are the means with standard deviation; n = 2 to 4 cells per subject's skin sample.

meation of Δ^9-THC, a greater resistance to diffusion was seen in the viable aqueous tissue layers (Challapalli & Stinchcomb 2002). Δ^8-THC would be expected to behave identically to Δ^9-THC, in regards to its permeation rate-limiting step, as both molecules have identical physicochemical properties except for the position of a double bond in the cyclohexene ring. Δ^9-THC and Δ^8-THC are more lipophilic than CBD and CBN, the former two drugs existing as oils at room temperature and the latter two as crystalline solids. All these drugs have similar molecular weights, so it stands to reason that the differences in permeabilities of these compounds are due to their lipophilicity differences (Potts & Guy 1992). Since Δ^9-THC and Δ^8-THC are more lipophilic than CBD and CBN, but have decreased permeabilities as compared to CBD and CBN, the viable tissue resistance to transport must be higher for Δ^9-THC and Δ^8-THC than for CBD and CBN. Therefore, increasing the polarity of the cannabinoids should improve their transdermal flux. Polarity will be a major consideration in the choice and/or design of future cannabinoids for transdermal dosage forms.

Identical formulations compared using receiver solutions of 0.5% Brij vs 4% BSA showed a significant difference in the case of CBN ($P < 0.05$), but not for Δ^8-THC or CBD. We did not see significant differences in cannabinoid drug diffusion study results when using 0.5% Brij vs 6% Brij (Challapalli & Stinchcomb 2002). The lag times for CBN were significantly longer than the CBD lag times ($P < 0.01$). Lag times for the 30–33% ethanol formulations were significantly reduced, as compared with the other vehicle treatments. No other statistically significant differences were identified among the flux data.

A significantly higher amount of drug was extracted from the skin at the end of the diffusion experiments for Δ^8-THC treatments as compared to CBN treatments ($P < 0.05$), and nearly a significantly higher level for Δ^8-THC as compared to CBD treatments ($P = 0.06$). This indicated that higher levels of the oil Δ^8-THC are miscible with the stratum corneum intercellular lipids than the crystalline CBD and CBN, or additionally/alternatively that Δ^8-THC is more significantly affected by viable-tissue controlled diffusion than CBD/CBN and more Δ^8-THC accumulates in the tissue. Comparison of the compounds' retention times

under the same reversed-phase HPLC conditions indicated that Δ^8-THC has the highest partition coefficient, followed by CBN, and then CBD. This order of hydrophobicity corresponds with the more polar CBD chemical structure, as CBD has the additional hydroxyl group as compared to Δ^8-THC and CBN. The order of hydrophobicity also corresponds with the skin tissue concentrations.

The decreased hydrophobicity of the CBD molecule, as compared to Δ^8-THC and CBN, should be in CBD's favour for improved flux since viable-tissue controlled diffusion is a significant participant in the transport mechanism of these compounds. Further transdermal formulation enhancement studies may reveal more about the transport mechanisms and potential differences among these closely related cannabinoid compounds.

Conclusions

The most significant amount of clinical cannabinoid data exists for Δ^9-THC, the active ingredient in the Marinol (dronabinol) capsule. A calculated required therapeutic delivery rate of Δ^9-THC can be estimated at about $9\,\text{nmol}\,\text{cm}^{-2}\,\text{h}^{-1}$, based on a clearance of $14\,\text{L}\,\text{h}^{-1}$ and a therapeutic plasma level of $10\,\text{ng}\,\text{mL}^{-1}$ (Weiner 1996). This might be similar to the expected delivery rate for Δ^8-THC, CBD and CBN, or perhaps less if drug combinations are used. The mean flux values ranged from 0.73 to $4.67\,\text{nmol}\,\text{cm}^{-2}\,\text{h}^{-1}$ for this group of experiments. Further permeation enhancer addition and patch formulation techniques will be investigated in order to increase the flux into the potential therapeutic delivery rate range. It is possible that higher flux rates may not be needed for each individual cannabinoid if combinations of cannabinoids provide synergistic therapeutic effects.

The extreme hydrophobicity of the cannabinoids make crossing the aqueous layer of the skin's viable tissue the rate-limiting step in the diffusion process for these drugs (Scheuplein & Blank 1973; Challapalli & Stinchcomb 2002). It is possible that in-vitro diffusion studies may provide a significant underestimate of the actual in-vivo transdermal flux because of the potential for lipophilic compounds to diffuse slowly through the aqueous environment of the dermis (Bronaugh 1996). Our experimental system membrane does contain a significant portion of dermis, although 10 times less than would be present in a full-thickness skin experiment. The essential piece of information for the cannabinoids' potential transdermal success will be the in-vivo experiments. These in-vivo experiments are currently underway in the hairless guinea pig model.

References

Bornheim, L. M., Kim, K. Y., Li, J., Perotti, B. Y. T., Benet, L. Z. (1995) Effect of cannabidiol pretreatment on the kinetics of tetrahydrocannabinol metabolites in mouse brain. *Drug Metab. Dispos.* **23**: 825–831

Bronaugh, R. L. (1996) *In vitro* viable skin model. In: Borchardt, R. T., Smith, P. L., Wilson, G. (eds) *Models for Assessing Drug Absorption and Metabolism*. Plenum Press, New York, pp 375–385

Challapalli, P. V. N., Stinchcomb, A. L. (2002) *In vitro* experiment optimization for measuring tetrahydrocannabinol skin permeation. *Int. J. Pharm.* **241**: 329–339

Consroe, P., Sandyk, R., Snider, S. R. (1986) Open label evaluation of cannabidiol in dystonic movement disorders. *Int. J. Neurosci.* **30**: 277–282

Cunha, J. M., Carlini, E. A., Pereira, A. E., Ramos, O. L., Pimentel, C., Gagliardi, R., Sanvito, W. L., Lander, N., Mechoulam, R. (1980) Chronic administration of cannabidiol to healthy volunteers and epileptic patients. *Pharmacology* **21**: 175–185

Dalton, W. S., Martz, R., Lemberger, L., Rodda, B. E., Forney, R. B. (1976) Influence of cannabidiol on delta-9-tetrahydrocannabinol effects. *Clin. Pharmacol. Ther.* **19**: 300–309

Fabin, B., Touitou, E. (1991) Localization of lipophilic molecules penetrating rat skin in vivo by quantitative autoradiography. *Int. J. Pharm.* **74**: 59–65

Grinspoon, L., Bakalar, J. B. (1997) *Marihuana, the Forbidden Medicine*. Yale University Press, New Haven, CT

Grotenhermen, F. (2003) Pharmacokinetics and pharmacodynamics of cannabinoids. *Clin. Pharmacokinet.* **42**: 327–360

GW Pharmaceuticals (online). Available from URL: http://www.gwpharm.com (accessed 2003 Sep 9)

Hildebrand, J. H., Scott, R. L. (1950) *The Solubility of Nonelectrolytes*. Reinhold Pub. Corp., New York, Chapter 12

Hollister, L. E., Gillespie, H. (1975) Interactions in man of delta-9-tetrahydrocannabinol II. Cannabinol and cannabidiol. *Clin. Pharmacol. Ther.* **18**: 80–83

Jaeger, W., Benet, L. Z., Bornheim, L. M. (1996) Inhibition of cyclosporine and tetrahydrocannabinol metabolism by cannabidiol in mouse and human microsomes. *Xenobiotica* **26**: 275–284

Karniol, I. G., Shirakawa, I., Kasinski, N., Pfeferman, A., Carlini, E. A. (1974) Cannabidiol interferes with the effects of Δ^9-tetrahydrocannabinol in man. *Eur. J. Pharmacol.* **28**: 172–177

Karniol, I. G., Shirakawa, I., Takahashi, R. N., Knobel, E., Musty, R. E. (1975) Effects of delta-9-tetrahydrocannabinol and cannabinol in man. *Pharmacology* **13**: 502–512

McArdle, K. E., Mackie, P. R., Hawksworth, G. M., Pertwee, R. G., Guy, G. W., Whittle, B. A. (2001) Human in vitro metabolism of Δ^9-tetrahydrocannabinol in the presence and absence of cannabidiol. Symposium on the Cannabinoids, 2001 Jun 28–30. San Lorenzo de El Escorial, International Cannabinoid Research Society, Burlington, p. 76

Mechoulam, R., Shvo, Y. (1963) The structure of cannabidiol. *Tetrahedron* **19**: 2073–2078

Petitet, F., Jeantaud, B., Rebaude, M., Imperato, A., Dubroeucq, M. C. (1998) Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of Δ^9 tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. *Life Sci.* **63**: PL1–6

Potts, R. O., Guy, R. H. (1992) Predicting skin permeability. *Pharm. Res.* **9**: 663–669

Sandyk, R., Snider, S. R., Consroe, P., Elias, S. M. (1986) Cannabidiol in dystonic movement disorders. *Psychiatry Res.* **18**: 291

Scheuplein, R. J., Blank, I. H. (1973) Mechanism of percutaneous absorption IV. Penetration of nonelectrolytes (alcohols) from aqueous solutions and from pure liquids. *J. Invest. Dermatol.* **60**: 286–296

Stinchcomb, A. L., Dua, R., Paliwal, A., Woodard, R. W., Flynn, G. L. (1995) A solubility and related physicochemical

property comparison of buprenorphine and its 3-alkyl esters. *Pharm. Res.* **12**: 1526–1529

Touitou, E., Fabin, B. (1988) Altered skin permeation of a highly lipophilic molecule: tetrahydrocannabinol. *Int. J. Pharm.* **43**: 17–22

Touitou, E., Fabin, B., Dany, S., Almog, S. (1988) Transdermal delivery of tetrahydrocannabinol. *Int. J. Pharm.* **43**: 9–15

Vinciguerra, V., Moore, T., Brennan, E. (1988) Inhalation marijuana as an antiemetic for cancer chemotherapy. *NY State J. Med.* **88**: 525–527

Weiner, B. (ed.) (1996) *Physician's Desk Reference Generics*, 2nd edn, Medical Economics, New Jersey, pp 1083–1086

Zajicek, J., Fox, P., Sanders, H., Wright, D., Vickery, J., Nunn, A., Thompson, A. (2003) Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomized placebo-controlled trial. *Lancet* **362**: 1517–1526

Zuardi, A. W., Shirakawa, I., Finkelfarb, E., Karniol, I. G. (1982) Action of cannabidiol on the anxiety and other effects produced by Δ^9-THC in normal subjects. *Psychopharmacology (Berl.)* **76**: 245–250

Zuardi, A. W., Morais, S. L., Guimarães, F. S., Mechoulam, R. (1995) Antipsychotic effect of cannabidiol. *J. Clin. Psychiatry* **56**: 485–486