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ORIGINAL ARTICLE

Cannabidiol bioavailability after nasal and transdermal application: effect of permeation enhancers

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Abstract

Context: The nonpsychoactive cannabinoid, cannabidiol (CBD), has great potential for the treatment of chronic and 'breakthrough' pain that may occur in certain conditions like cancer. To fulfill this goal, suitable noninvasive drug delivery systems need to be developed for CBD. Chronic pain relief can be best achieved through the transdermal route, whereas 'breakthrough' pain can be best alleviated with intranasal (IN) delivery. Combining IN and transdermal delivery for CBD may serve to provide patient needs-driven treatment in the form of a nonaddictive nonopioid therapy. *Objective:* Herein we have evaluated the IN and transdermal delivery of CBD with and without permeation enhancers. *Materials and Methods:* In vivo studies in rats and guinea pigs were carried out to assess nasal and transdermal permeation, respectively. *Results:* CBD was absorbed intranasally within 10 minutes with a bioavailability of 34–46%, except with 100% polyethylene glycol formulation in rats. Bioavailability did not improve with enhancers. The steady-state plasma concentration of CBD in guinea pigs after transdermal gel application was 6.3 ± 2.1 ng/mL, which was attained at 15.5 ± 11.7 hours. The achievement of a significant steady-state plasma concentration indicates that CBD is useful for chronic pain treatment through this route of administration. The steady-state concentration increased by 3.7-fold in the presence of enhancer. A good in vitro and in vivo correlation existed for transdermal studies. *Conclusion:* The results of this study indicated that CBD could be successfully delivered through the IN and transdermal routes.

Key words: *Cannabidiol; cannabinoids; guinea pig; in vitro studies; in vivo studies; intranasal; pharmacokinetics; rat; transdermal*

Introduction

Some cannabinoids are potentially useful in cancer symptom management to alleviate pain and control chemotherapy-induced nausea and vomiting and to stimulate appetite and weight gain in AIDS and cancer patients. Cannabidiol (CBD) (Figure 1) may be a clinical candidate for the management of medical conditions where emesis and pain are prevalent[1,2]. There have been publications of several important clinical trials during the recent years, which point to the potential role of CBD in pain management. Cannabinoid treatments, specifically a combination of CBD and Δ^9-tetrahydrocannabinol (THC) extracts, have significantly reduced patient pain scores of acute pain in postoperative clinical trials[3] and of chronic pain associated with multiple sclerosis, peripheral neuropathy, rheumatoid arthritis, and central neuropathic pain[4–6]. GW Pharmaceutical's 'Sativex®', an oromucosal spray composed primarily of THC and CBD (1:1 ratio), has been approved in Canada for the symptomatic relief of neuropathic pain in multiple sclerosis and as an adjunctive analgesic treatment in patients with advanced cancer. Thus, CBD may be a potential candidate for the treatment of chronic and acute 'breakthrough' pain in the future.

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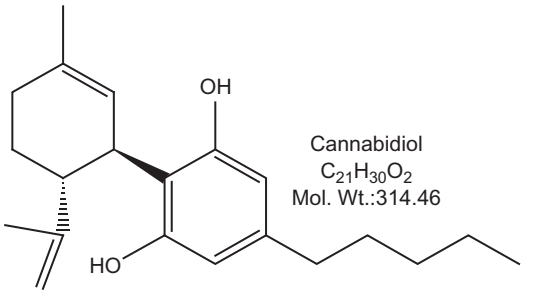

Figure 1. Chemical structure of CBD.

Oral bioavailability of CBD is low and ranges from 6% to 33% in humans and dogs, respectively[7–9]; hence the oral route is not ideal for the therapeutic delivery of CBD. As the drug has low aqueous solubility and undergoes first-pass metabolism, alternative delivery routes are needed to achieve successful therapeutic effects by bypassing the first-pass effect. A feasible method of CBD administration in patients for chronic pain relief would be through the transdermal route, thus maintaining a constant therapeutic drug level via zero-order delivery and reducing increased side effects because of the decreased peak plasma levels. Another potential route of systemic administration of CBD for acute or breakthrough pain would be through intranasal (IN) administration, which results in the rapid attainment of the drug blood level. IN delivery is superior to a drug injection because it is a noninvasive pain-free treatment that can improve patient compliance. Development of a nasal formulation of CBD would help to treat possible breakthrough pain and nausea bouts experienced while on transdermal therapy, providing rapid increases in drug levels when symptoms are exceptionally magnified. There is no evidence anywhere in the literature that a 'rescue' cannabinoid nasal formulation has been used with a chronic controlled-release cannabinoid dosage form. This idea is typical of opiate pain therapy, where patients may wear fentanyl patches for chronic pain and use fentanyl lozenges for 'breakthrough' pain. With multiple literature reports describing a reduced need for opiates with the combination of opiate/cannabinoid treatment, it may also be possible to use a 'rescue' cannabinoid nasal spray with controlled-release opiate therapy[10–12]. Thus, a 'rescue' IN formulation of CBD may have the potential to be used in combination with various controlled-release pain formulations to provide a patient-centric approach.

It has been demonstrated, although with only a few published reports, that cannabinoids are good drug candidates for transdermal delivery in the treatment of chronic conditions[13–19]. Only two articles deal with the transdermal delivery of CBD[20,21]. Stinchcomb et al.[20] evaluated the in vitro permeation of CBD in human skin, whereas Lodzkia et al.[21] reported the in vivo transdermal delivery of CBD in a mouse model. The use of permeation enhancers to increase the permeation of CBD in vitro has also been described in one patent application[22]. Articles describing the IN administration of cannabinoids are also very few. Our laboratory has recently assessed the nasal bioavailability of two cannabinoids, Δ^9-THC and WIN 55212[23,24]. One patent describes IN Δ^9-THC prodrugs[25]. There is no scientific literature on nasal pharmacokinetics and bioavailability of CBD. Hence, the main objective of this article is to investigate the IN permeation of CBD in an anesthetized rat nasal absorption model[26] and transdermal permeation in a hairless guinea pig model. The most likely pitfall to be encountered in the attempt to achieve therapeutic levels of the cannabinoids transdermally and intranasally is the extreme lipophilicity of these compounds, which may make traversing the aqueous media of the skin's/nasal viable tissue arduous. Hence, various formulations with or without the addition of enhancers were assessed in this study.

Materials and methods

Chemicals and films

CBD was a generous gift from the National Institute on Drug Abuse (Research Triangle Park, NC, USA). Propylene glycol (PG), USP, and sodium glycocholate were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Dimethyl-β-cyclodextrin was purchased from Acros (Morris Plains, NJ, USA). Ethanol was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). Polyethylene glycol (PEG) 400 was purchased from EMD chemicals Inc. (Gibbstown, NJ, USA). Hydroxyethyl cellulose 250HHXPHARM (Natrasol®) was obtained as a free sample from Hercules Incorporated (Wilmington, DE, USA). Transcutol HP (diethylene glycol monoethyl ether EP/USP-NF) was obtained from Gattefosse (Cedex, France). Ammonium acetate, ethyl acetate, and acetonitrile (ACN) were obtained from Fisher Scientific (Fairlawn, NJ, USA). Ammonium citrate was obtained from Alfa Aesar (Ward Hill, MA, USA). ARcare® 7396 (pressure-sensitive tape with MA-38 medical grade acrylic adhesive and 60# Kraft release paper) was obtained from Adhesives Research, Inc. (Glen Rock, PA, USA). Scotch™, Scotchpak™ 1109 SPAK 1.34 mL heat-sealable polyester film, and Scotchpak™ 1022 release liner 3.0 mL were obtained from 3M™ Drug Delivery Systems (St. Paul, MN, USA). Water was purified by a Barnstead nanopure Diamond™ Ultrapure water system (Barnstead International, Dubuque, IA, USA). All the glassware used in the study was silanized, and siliconized microcentrifuge tubes were used (Fischer Scientific, Pittsburgh, PA, USA).

IN studies in rats

Preparation of intravenous and IN formulations of CBD

For IN administration, a group of rats were dosed with CBD formulation in PEG 400 alone (PEG 400-A). Another group was dosed with CBD in 50:35:15 (v/v) PEG:saline:ethanol solvent system (PEG 400-ES) with the enhancers, 1% sodium glycocholate (PEG 400-ES+1% glycocholate) or 1% (w/v) DM-β-CD (PEG 400-ES+1% DM-β-CD), or without any enhancer. CBD was first dissolved in PEG 400 (50% of the total volume), the solution was vortexed and sonicated for 5 minutes. The volume was then made up with PEG 400 or a mixture of sterile saline and ethanol (7:3), with or without the enhancers, to give CBD concentration of 2.8 mg/mL. The solution was vortexed and sonicated for 5 minutes. For intravenous (IV) administration, the formulation PEG 400-ES, was further diluted to inject about 170 µL (200 µg/kg) of the drug. Drug solutions were prepared immediately before each animal was dosed. IV formulation based on PEG 400-ES was needed to compare the pharmacokinetic parameters of PEG 400-ES with or without enhancers after IN dosing, as CBD is not soluble at a sufficient concentration in saline alone.

Animal studies

All animal studies were in accordance with institutional guidelines approved by the University of Kentucky IACUC. All animal procedures were conducted according to the Principles of Laboratory Animal Care (NIH publication no. 85–23). The pharmacokinetics of CBD in anesthetized rats was determined after IV (200 µg/kg) and IN (200 µg/kg) administration. For IV administration, the right jugular vein and the left femoral vein were cannulated for blood sampling and drug administration, respectively. Cannula was flushed with the vehicle followed by normal saline immediately after IV dosing. For IN study, animals (three rats per group) were prepared for pharmacokinetic studies using a surgical procedure allowing IN administration described previously[26]. Ten microliters of the drug was instilled in each nostril. Blood samples were collected from the jugular vein of each animal before drug treatment (baseline) and at regular time intervals until 4 hours after IV and IN doses. Samples were transferred to 1.5-mL siliconized microcentrifuge tubes containing 5 µL heparin (500 U/mL). The samples were immediately centrifuged (10,000 × *g* for 3 minutes) and the collected plasma samples were stored at −70°C. Drug was extracted from the plasma samples using a single-step precipitation–extraction procedure and quantitated using high-pressure liquid chromatography (HPLC) with mass spectrometry (MS) detection.

Transdermal studies in hairless guinea pigs

Preparation of IV and transdermal gel formulation of CBD

CBD was prepared in sterile saline with 3% Tween 80 and 5% PG for IV administration to hairless guinea pigs. The gel formulation for transdermal delivery was prepared by making a solution of 80:20 PG:nanopure water. CBD was weighed out and the appropriate volume of solution was calculated to give an 18 mg/mL solution. From this solution, 6% was removed and replaced with Transcutol HP for the enhancer study. Transcutol HP at 6% (v/v) was used because this concentration has been shown to enhance the steady-state flux of CBD by 2.41-fold in vitro as compared to that of CBD alone[22]. The solution was weighed to determine the amount of the gelling agent, hydroxyethyl cellulose (2% for CBD alone and 5% for enhancer formulation), needed. A higher concentration of hydroxyethyl cellulose in the formulation with the enhancer was needed to make the gel of similar consistency to that without the enhancer. A slight difference in the viscosity between the gels, if any, is not expected to affect the passive transdermal permeation of CBD. The gelling agent was weighed and slowly added to the drug solution. The drug solution was vortexed for 30 seconds between each addition of gelling agent. The drug solution was sonicated for 15 minutes, removing the solution periodically to vortex.

Preparation of patches

Patches were constructed by applying Scotch™ permanent double-sided tape to the top of a flat rubber ring (2 mm thick) with an inner diameter of 28 mm. A piece of Scotchpak™ 1109 SPAK 1.34 mL heat-sealable polyester film was attached to the rubber ring with the tape. On the other side of the ring, ARcare® 7396 was applied. The backing of the ARcare® 7396 was removed and the patches were placed on Scotchpak™ 1022 release liner 3.0 mL. Figure 2 shows the patch and the patch being taken off the guinea pig.

Animal studies

All animal studies were in accordance with institutional guidelines approved by the University of Kentucky IACUC. All animal procedures were conducted according to the Principles of Laboratory Animal Care (NIH publication no. 85–23). Male and female hairless IAF and Hartley guinea pigs (Charles River) weighing 350–450 g were used for these studies. Surgical procedures were carried out to cannulate the jugular vein. Blood samples were collected from each animal before drug treatment (baseline). For transdermal studies, 500 µL of the CBD gel formulation with or without the enhancer, Transcutol HP, was applied on the dorsal region of the

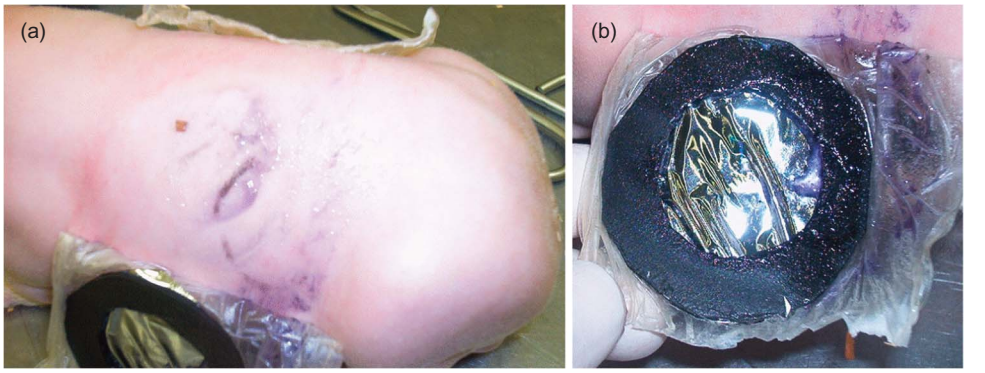

Figure 2. (a) Transdermal patch being taken off the guinea pig. (b) The patch used in the transdermal study.

hairless guinea pigs on both sides, and a patch was placed over the solution for containment. Bioclusive tape was applied over the two patches followed by a protective stocking. Patches remained on the guinea pig for 48 hours and were removed. After patch removal, excess gel solution was cleaned off the guinea pig's skin. Blood samples were obtained from jugular cannulas over the 48 hours treatment period and for 48 hours post-dosing. Guinea pigs were also dosed IV with 1 mg/kg of CBD followed by flushing with vehicle and saline. Blood samples collected at regular time intervals were centrifuged at 10,000 × *g* for 3 minutes to harvest plasma for LC–MS analysis. Guinea pig's weight, rectal temperature, and feed intake were monitored daily.

Analytical method for CBD in plasma samples

Plasma sample extraction procedure

Plasma sample of 0.05 mL was extracted with 500 µL of ACN:ethyl acetate (1:1, v/v). The mixture was vortexed for 30 seconds and centrifuged at 10,000 × *g* for 20 minutes. The supernatant was pipetted into a 3 mL silanized glass test tube and evaporated under nitrogen at 37°C. The residue was reconstituted with 100 µL of ACN, vortexed, and sonicated for 5 minutes. The samples were transferred to autosampler vials containing silanized low volume inserts and 20 µL was injected onto the HPLC column. The extraction efficiency was 93 ± 4%.

Liquid chromatography

Chromatography was performed on a Waters Symmetry® C_{18} (2.1 × 150 mm, 5 µm) column at 35°C with a mobile phase consisting of ammonium acetate (2 mM):ACN (20:80, v/v) at a flow rate of 0.25 mL/min.

Mass spectrometry detection

HPLC with MS detection analyses were carried out utilizing a Waters Alliance 2695 pump and autosampler and a Micromass ZQ detector (Milford, MA, USA) using electrospray ionization for ion production. Selected ion monitoring was performed in the negative ion mode for CBD, m/z 313 (dwell time 0.30 second). The capillary voltage was 3.5 kV and the cone voltage was 40 V. The source block and the desolvation temperatures were 120°C and 250°C, respectively. Nitrogen was used as a nebulization and drying gas at flow rates of 50 and 450 L/h, respectively. The retention time for CBD was between 5.34 and 5.4 minutes and the LLQQ was 1.25 ng/mL.

Data treatment

Pharmacokinetic analysis of CBD plasma concentrations versus time profiles after IV bolus and IN administration in rats was carried out by fitting the data to a three-compartment and one-compartment model with absorption, respectively (WinNonlin Professional, version 4.0, Pharsight Corporation, Mountain View, CA, USA).

The data generated after IV dosing in hairless guinea pigs were analyzed with a two-compartment model using WinNonlin®. Transdermal data were analyzed by a noncompartmental method.

Statistical analysis of the in vivo data from IV, IN, and transdermal gel application was performed by one-way analysis of variance followed by Tukey's post hoc analysis using SigmaStat (SPSS Inc., Chicago, IL, USA). The level of significance was set at $P < 0.05$. Data represent mean ± SD.

Results and discussion

IN studies in rats

IV studies in rats were carried out to compare the IN absorption and to estimate the absolute bioavailability of CBD. The mean observed and the predicted plasma drug concentration–time profile after single IV bolus

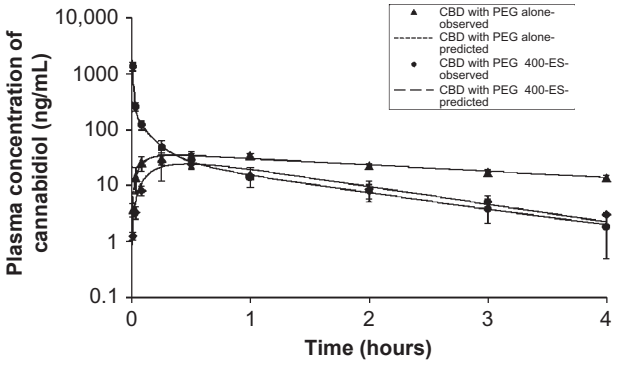

Figure 3. Plasma concentration time profiles of CBD 200 μg/kg after IV (n = 4) and IN (n = 3) administration in rats. Data represent mean ± SD.

administration of CBD together with nasal absorption profiles are illustrated in Figure 3. The plasma levels of CBD after IV and IN administration were adequately described using a three-compartment open model and a one-compartment model with absorption phase, respectively. The pharmacokinetic parameters of CBD after IV and IN administration are summarized in Table 1.

The maximum plasma concentration of CBD in rats after IV administration was 3.6 ± 1.5 μg/mL. The plasma concentration declined rapidly to an average of 18.9 ng/mL at about 1 hour and 9 ng/mL at 2 hours. The mean steady-state volume of distribution was 7.59 ± 1.57 L/kg, which is lower than the highly lipophilic Δ^9-THC (32.20 ± 18.94 L/kg) in rats[24], as expected because of the more polar nature of CBD. CBD has a threefold higher clearance (10.21 ± 2.75 versus 3.37 ± 1.47 L/h) and shorter terminal elimination half-life (1.06 ± 0.42 versus 7.27 ± 1.95 hours) compared to Δ^9-THC, suggesting that CBD is eliminated faster in rats.

The nasal absorption of CBD from all formulations was rapid ($T_{max} \leq 10$ minutes). CBD was detected in the first sample taken at 0.5 minutes after IN administration of all the treatments indicating rapid absorption through the nasal epithelium. Figure 3 shows the plasma concentration–time course of CBD (200 μg/kg dose) after IN dosing with formulations containing PEG 400-A and PEG 400-ES compared to the IV dose profile. The absorption of CBD from PEG 400-A was faster than that from PEG 400-ES ($t_{1/2}$ = 0.06 ± 0.02 versus 0.14 ± 0.09 hour; no significant difference) whereas elimination was slower ($t_{1/2}$ = 2.78 ± 0.67 versus 1.31 ± 0.33 hours; $P < 0.05$). PEG 400-A resulted in a 3.5-fold increase in area under the curve (AUC) and more than double the extent of absorption compared to PEG 400-ES, whereas C_{max}, T_{max}, and CL were not affected (Table 1). The difference in AUC between the two formulations can be attributed to a very high amount of PEG used in PEG 400-A formulation.

Sodium glycocholate, a bile salt, has been used for nasal absorption enhancement of both hydrophilic peptides[27] and lipophilic compounds like diazepam[28]. Sodium glycocholate may act by altering the drug solubility, drug partition coefficient, and/or weak ionic interactions with the drugs and also by interacting with the membrane lipid and opening the tight junctions[29,30]. Methylated cyclodextrins have also been shown to be important absorption enhancers for nasal delivery of different classes of compounds[31]. The permeability enhancing effect of cyclodextrins is because of their interaction with the nasal epithelial membranes and their ability to transiently open tight junctions[32]. One percent sodium glycocholate[27] and up to 2% methylated cyclodextrins[31] have been used for nasal drug delivery without any detrimental effects. Hence, these chemicals were used in this study to investigate their enhancing effect on the nasal absorption of CBD.

Table 1. Pharmacokinetic parameters of CBD after IV (200 μg/kg) and IN (200 μg/kg) administration in rats.

	Intravenous[a]	Intranasal[b]			
Pharmacokinetic parameters	PEG 400-ES	PEG 400-A	PEG 400-ES	PEG 400-ES + 1% Glycocholate	PEG 400-ES + 1% DM-β-CD
AUC (ng/mL/h)	103.56 ± 27.73	151.63 ± 18.45	43.26 ± 11.38	41.76 ± 3.06	34.59 ± 3.50
Absorption half-life (hours)	—	0.06 ± 0.02	0.14 ± 0.09	0.13 ± 0.02	0.07 ± 0.02
Elimination half-life (hours)	1.06 ± 0.42	2.78 ± 0.67	1.31 ± 0.33	1.21 ± 0.24	1.39 ± 0.28
CL (L/h/kg)	10.21 ± 2.75	6.66 ± 0.84	4.83 ± 1.18	4.81 ± 0.37	5.82 ± 0.61
T_{max} (hours)	—	0.35 ± 0.13	0.48 ± 0.16	0.47 ± 0.08	0.33 ± 0.05
C_{max} (ng/mL)	3596 ± 1497	35.39 ± 5.88	27.35 ± 1.52	30.23 ± 1.53	19.90 ± 1.1
V_{ss} (L/kg)	7.59 ± 1.57	—	—	—	—
F (%)	—	1.4 ± 0.2	0.46 ± 0.11	0.43 ± 0.03	0.34 ± 0.04

AUC, area under the curve; CL, systemic clearance; C_{max}, maximum plasma concentration; T_{max}, time to attain maximum plasma concentration; F, bioavailability; V_{ss}, steady-state volume of distribution; PEG 400-A, polyethylene glycol alone; PEG 400-ES, polyethylene glycol:saline:ethanol (50:35:15). Data represent mean ± SD.

[a] n = 4.

[b] n = 3.

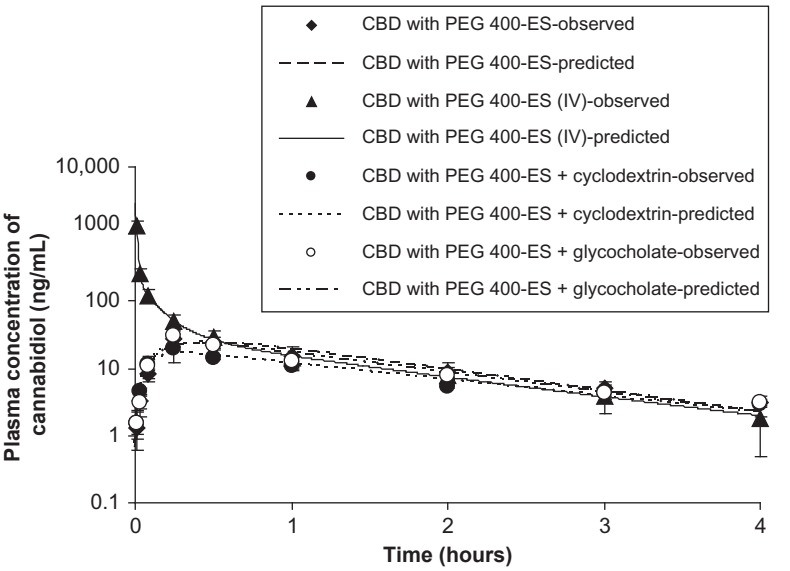

Figure 4. Plasma concentration time profiles of various CBD formulations after IN administration in rats (200 μg/kg; $n = 3$). Data represent mean ± SD.

The plasma profiles of CBD after IN dosing with formulations containing PEG 400-ES with 1% DM-β-CD or 1% glycocholate are shown in Figure 4. The AUC of the CBD formulations with glycocholate (41.76 ± 3.06 ng/mL/h) or DM-β-CD (34.59 ± 3.5 ng/mL/h) was comparable to that of PEG 400-ES formulation (43.26 ± 11.38 ng/mL/h). The inclusion of 1% glycocholate did not alter the absorption half-life, C_{max}, T_{max}, clearance, and extent of absorption of CBD ($P > 0.05\%$) (Table 1). Inclusion of DM-β-CD, however, had a tendency to cause a rapid absorption of CBD as evidenced by the absorption half-life and T_{max} values but without a significant difference compared to other formulations (Table 1). It did not result in the enhancement of AUC, C_{max}, or the bioavailability of CBD; rather it tended to decrease the value of these parameters though not significantly. This tendency was also observed with WIN 55212-2, a synthetic cannabinoid, in a previous study carried out in our laboratory with various cyclodextrins[23]. One possible explanation for this observation may be poor or nonsurfactant properties of the cyclodextrins, as compared to sodium glycocholate, or the rapid precipitation of the cyclodextrin formulation in the nasal fluids. The other reason may be the high concentration of PG or PEG 400 in the formulations, which possibly interferes with the absorption enhancement mechanisms of cyclodextrins. The fact that, in addition to cyclodextrins, sodium glycocholate also did not enhance the absorption of CBD may be because addition of the enhancers reduces the thermodynamic activity of CBD by increasing its saturation concentration, thus decreasing the penetration rate.

One of the biggest concerns in IN formulations is the sensitivity of the nasal mucosa to the solvents and other additives in the formulations. PEG was used as a vehicle in this study because it has been found to exert only mild local toxicity to the nasal mucosa[33,34] and thus may be less irritating compared to other glycols like PG. PEG has been included in many nasal sprays up to a concentration of 15% and has been used in clinical situation. However, because of the high proportion of PEG used in this study, a more suitable formulation of CBD containing a lower PEG amount needs to be evaluated.

In this study, CBD absolute bioavailabilities achieved with different nasal formulations were in the range of 34–46%. The absolute bioavailabilities of WIN 55212-2[23] and Δ^9-THC[24] in our previous studies ranged between 61–77% and 6.4–9.1%, respectively. Disregarding the anesthetized rat model used in these studies, which somewhat overestimates the absolute bioavailability, and the various formulations used, the rank order of the bioavailabilities of the compounds correlated with their lipophilicities (cLog P = Δ^9-THC, 7.238; CBD, 6.641; WIN 55212-2, 4.28). The bioavailability decreased with increasing lipophilicity of the compound. This emphasizes the need of a more polar formulation of CBD to increase the nasal permeation in the presence of epithelial mucus and other polar secretions.

Transdermal studies in hairless guinea pigs

The mean plasma concentrations obtained with CBD gel without the enhancer on hairless guinea pigs are presented in Figure 5, and a summary of the pharmacokinetic

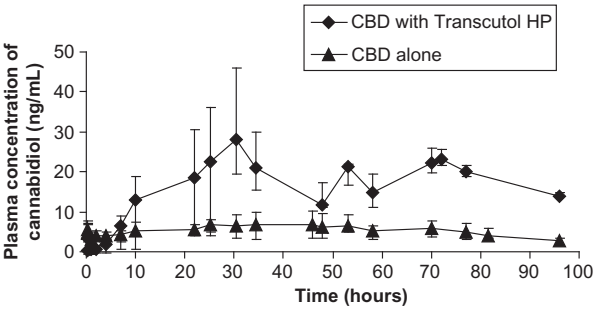

Figure 5. Plasma concentration time profiles of CBD after application of gel formulation in hairless guinea pigs. Data represent mean ± SD, $n = 6$ (without enhancer), and $n = 3$ (with enhancer).

Table 2. Pharmacokinetic parameters of CBD after transdermal gel application in hairless guinea pigs.

Pharmacokinetic parameter	CBD alone[a]	With Transcutol-HP[b]
AUC_{0-48} (ng/mL/h)	276 ± 93	888 ± 419
C_{max} (ng/mL)	8.6 ± 2.5	35.6 ± 11.6
T_{max} (hours)	38.4 ± 19.2	31.2 ± 29.4
C_{ss} (ng/mL)	6.3 ± 2.1	23.7 ± 7.3
T_{lag} (hours)	15.5 ± 11.7	23.6 ± 2.33
Enhancement factor	—	3.7

Data represent mean ± SD.
[a]$n = 6$.
[b]$n = 3$.

parameters is given in Table 2. The results of the in vivo studies showed that the steady-state plasma concentrations of CBD were 6.3 ± 2.1 ng/mL, which were attained at 15.5 ± 11.7 hours. The steady-state plasma concentrations were maintained throughout the 48 hours of gel application and started declining at about 6 hours after gel removal, indicating a skin reservoir effect. The skin reservoir property of CBD is because of the high lipophilic property of the drug that makes it easier to cross the stratum corneum but makes it difficult to traverse through the aqueous dermis. This was reflected in high accumulation in the skin and a relatively lower flux of lipophilic drugs in the previous in vitro experiments[20]. Δ^8-THC, which is more lipophilic than CBD, also exhibited skin reservoir properties in guinea pigs in vivo, the steady-state level lasting for about 70 hours after initiating treatment with the drug[17]. The steady-state plasma concentrations of CBD in guinea pigs (gel; 6.3 ng/mL) were higher and lower than the steady-state levels achieved with Δ^8-THC (patch; 4.4 ng/mL)[17] and WIN 55212-2 (patch; 8.6 ng/mL)[19], respectively. With the use of the enhancer, Transcutol HP, at 6% (v/v) in the gel formulation, the plasma concentration of CBD was significantly enhanced by 3.7-fold (Figure 5). Transcutol acts as a solubilizing agent and is suitable because it is nontoxic and biocompatible with the skin[35]. Transcutol enhances the skin permeation rate of various drugs[36–38]. This effect is more significant when Transcutol is used in combination with suitable cosolvents[37,39,40]. The use of PG in this study may have a possible synergistic enhancer effect.

A blood concentration of 10 ng/mL similar to that of Δ^9-THC[41] may be considered therapeutic for CBD because both molecules share similar chemical structures and pharmacokinetics. A plasma level of 6–11 ng/mL was achieved in a double-blind, placebo-controlled, randomized crossover trial of oral CBD administration (10 mg/kg/day) for 6 weeks in patients with Huntington disease[42]. Although substantial levels of CBD could be delivered through the transdermal route in this study, the level attained would be subtherapeutic considering a comparable permeability between guinea pig skin and human skin for cannabinoid permeation. Previous in vitro studies with human skin using PG:Hanks' buffer (4:1) as donor solution have shown that the steady-state flux and cumulative amount of CBD permeated could be increased by 2.4-fold with the use of Transcutol HP[22]. The difference in enhancement between this in vitro human skin study and our current in vivo study in guinea pigs was only about 1.3-fold. Our previous data with other cannabinoids have also shown that hairless guinea pig is the ideal model for testing cannabinoid transdermal systems, as we observed no difference to 1.2-fold differences in drug permeability between hairless guinea pig and human skin in vitro[17,18]. Specifically, there was no significant difference observed in the flux, lag times, or drug content in the skin of the guinea pig compared to human skin in the in vitro studies of Δ^8-THC[17]. The observed steady-state plasma concentration of Δ^8-THC (4.3 ng/mL) was in very good agreement with the predicted plasma concentration of Δ^8-THC (4.3 ng/mL). Similarly, the in vitro transdermal flux of WIN 55212-2 through human skin and guinea pig skin was found to be 650 ± 23 ng/cm^2/h and 777 ± 29 ng/cm^2/h, respectively[18]. There was a significant difference in the flux through human skin versus guinea pig skin. However, this difference in the permeability of WIN 55212-2 between guinea pig and human skin was only a factor of 1.2, so guinea pig skin could be used as an alternative to human skin in in vitro studies because the drug permeability was very close. The observed in vivo results in the guinea pig were in excellent agreement with the predicted plasma concentrations from the in vitro data.

The predicted steady-state plasma concentrations of CBD in the guinea pigs following the gel application

were calculated from the in vitro steady-state flux using the following equation:

$$C_{ss} = \frac{J_{ss}A}{\mathrm{CL}}, \quad (1)$$

where 'C_{ss}' is the predicted steady-state plasma concentration (ng/mL), 'J_{ss}' is the steady-state flux across human skin (see discussion above), 'A' is area of the applied gel (13.2 cm^2), and 'CL' is the total clearance in guinea pigs.

IV studies of CBD (1 mg/kg) were carried out in hairless guinea pigs to obtain the pharmacokinetic parameter, systemic clearance, CL, for the prediction of steady-state concentration in the guinea pigs. The mean observed and predicted plasma drug concentration–time profile after IV bolus administration of CBD is given in Figure 6. Plasma CBD levels declined over time in a biexponential manner; a two-compartment open model was chosen to best fit the data. The pharmacokinetic parameters are summarized in Table 3. The mean maximum plasma concentration of CBD after IV administration was 269 ng/mL. The plasma concentration declined rapidly to an average of 33 ng/mL at about 1 hour and 15 ng/mL at 2 hours. The mean steady-state volume of distribution, systemic clearance, and terminal elimination half-life was 13.7 ± 4 L/kg, 3.2 ± 0.8 L/h, and 3.5 ± 2 hours, respectively. The mean steady-state volume of distribution was higher, systemic clearance was lower, and elimination half-life was longer when compared to the pharmacokinetic parameters in the rats (Table 1).

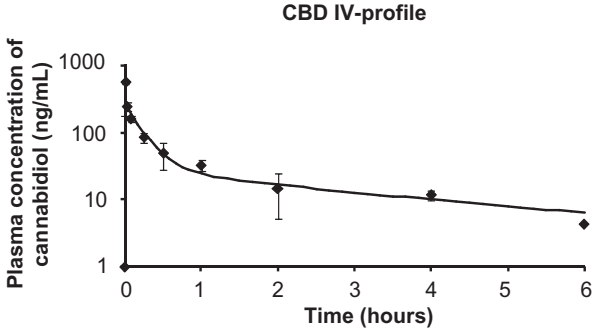

Figure 6. Mean (±SD) plasma profile after IV administration of CBD (1 mg/kg) in hairless guinea pigs ($n = 6$).

Table 3. Pharmacokinetic parameters of CBD after IV administration (1 mg/kg) in hairless guinea pigs ($n = 6$).

Parameter	CBD
AUC (ng/mL/h)	175 ± 60
α (h^{-1})	4.75 ± 1.07
β (h^{-1})	0.25 ± 0.12
$t_{1/2}\,\alpha$ (hours)	0.15 ± 0.03
$t_{1/2}\,\beta$ (hours)	3.47 ± 1.99
C_{max} (ng/mL)	269 ± 70
CL (L/h/kg)	3.22 ± 0.78
V_{ss} (L/kg)	13.72 ± 3.99

Data represent mean ± SD.

The steady-state flux (J_{ss}) value was taken from our previous in vitro human skin permeation studies with CBD[22]. In brief, this study was carried out using a PermeGear flow-through (In-Line, Riegelsville, PA, USA) diffusion cell system. The receiver solution was HEPES-buffered Hanks' balanced salts with gentamicin (containing 40% PEG 400, pH 7.4), and the flow rate was adjusted to 1.1 mL/h. Each cell was charged with 0.25 mL of the donor solution [an excess quantity of CBD in PG:Hanks' buffer (4:1) solution with and without permeation enhancers at 6% (v/v)]. Samples were collected in 6-hour increments for 48 hours and analyzed using HPLC. Experiments were carried out in three cells ($n = 3$) from the same donor. The flux of CBD alone and with Transcutol was 6.13 ± 0.43 and 14.81 ± 1.08 nmol/cm^2/h, respectively. Considering the similar flux of CBD in human and guinea pig skin (see discussion above), these flux values may be taken into account for calculating the predicted steady-state concentration in guinea pigs.

A good correlation between the in vitro and the present in vivo studies of CBD was obtained. The predicted plasma concentration in guinea pigs was 7.9 ± 0.55 and 19.09 ± 1.39 ng/mL of CBD alone and with Transcutol HP, respectively. These values were calculated from Equation (1) using IV clearance value of 3.22 ± 0.78 L/h in guinea pigs and drug application area of 13.2 cm^2. There was no significant difference between the observed and predicted plasma concentrations of CBD either alone or with Transcutol HP. This further emphasizes that in vitro experiments with guinea pig skin may be used for transdermal drug delivery studies with cannabinoids.

Conclusion

The aim of this study was to examine the IN and transdermal delivery potential of CBD. IN application of CBD formulations resulted in relatively rapid and significant absorption of CBD from the nasal cavity. Bioavailability of CBD decreased when the formulation content of PEG 400 decreased from 100% (PEG 400-A) to 50% (PEG 400-ES). Addition of sodium glycocholate or DM-β-CD to the 50% PEG formulation of CBD (PEG 400-ES) did not result in increased permeation. CBD provided significant plasma drug levels after topical gel application

in vivo. Transcutol HP enhanced the CBD steady-state plasma concentration by 3.7-fold. A good correlation existed between in vitro and in vivo results. Future studies will include optimization of the nasal and transdermal drug formulations for achievement of more rapid and higher plasma drug levels, respectively.

The major problems associated with psychoactive cannabinoids are the abuse potential and other central nervous system side effects seen in patients at higher oral doses. CBD, by virtue of its nonpsychoactive property, should be devoid of such effects. This is especially important to avoid after IN delivery, which may result in high concentrations in the brain through rapid absorption. A potential low-dose nasal formulation that would be intended to be used with a transdermal system of the drug would also avoid the need for high CBD concentrations in the nasal formulation. The safety profile of the CBD transdermal system would also be improved because the plasma drug levels rise slowly, causing any peak-drug level-related side effects to be curbed. Given the need for a nonaddictive and effective alternative treatment for breakthrough and chronic pain for those who do not respond well to the currently available medications, the future direction of CBD research should focus on the development of alternative dosage forms.

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Declaration of interest

Dana C. Hammell and Audra L. Stinchcomb are shareholders in AllTranz Inc., a specialty pharmaceutical company developing a CBD gel product.

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