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Pharmacokinetics and Tolerability of Δ^9-THC-Hemisuccinate in a Suppository Formulation as an Alternative to Capsules for the Systemic Delivery of Δ^9-THC

Mahmoud A. ElSohly[a–c] Waseem Gul[a, b] Larry A. Walker[a, b]

[a]ElSohly Laboratories, Inc., Oxford, MS, USA; [b]National Center for Natural Products Research (NCNPR), School of Pharmacy, University of Mississippi, Oxford, MS, USA; [c]Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, Oxford, MS, USA

Keywords

Cannabinoids · Δ^9-THC · Pharmacokinetics · THC metabolites · THC prodrugs · Suppository formulations

Abstract

The objectives of this study were: (1) to assess the safety, tolerability, and pharmacokinetics of ascending doses of Δ^9-tetrahydrocannabinol-hemisuccinate (THC-HS) after rectal administration as suppositories in male volunteers; and (2) to compare the pharmacokinetics of oral administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) with an equivalent amount of Δ^9-THC delivered as THC-HS via the suppository formulation. In support of the pharmacokinetic evaluations, an analytical method was developed and validated for the determination of Δ^9-THC and for its major circulating metabolites 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in human plasma. Δ^9-THC, 11-OH-THC, and THC-COOH were extracted from plasma using solid phase extraction and analyzed by liquid chromatography-tandem mass spectrometry. The limits of detection and quantitation for all 3 analytes were 0.25 and 0.5 ng/mL, respectively. The method was validated over the range of 0.5–25 ng/mL. This method was used to quantify Δ^9-THC and any THC-HS as Δ^9-THC due to the inclusion of a hydrolysis step as part of the extraction procedure. Therefore, Δ^9-THC measured was the total THC (free Δ^9-THC plus Δ^9-THC derived from THC-HS). The assay was reproducible for the measurement of all 3 analytes, with a variability of 7.2, 13.7, and 8.3%, respectively, at the 1 ng/mL level. The method was then used to assess the pharmacokinetics of Δ^9-THC and metabolites from the suppository dosage form in doses equivalent to 1.25, 2.5, 5, 10, and 20 mg Δ^9-THC per suppository as THC-HS. Systemic exposure to Δ^9-THC, administered as THC-HS suppository, increased broadly dose proportionally. Systemic exposure and $C_{max\ (obs)}$ estimates for 11-OH-THC and THC-COOH generally increased subproportionally. The pharmacokinetic profiles of Δ^9-THC and metabolites were also compared after oral administration of 10 mg Δ^9-THC (as dronabinol capsules) and after administration of 10 mg equivalents of Δ^9-THC as THC-HS in suppository form. Total systemic exposure to Δ^9-THC was considerably higher following rectal administration of THC-HS than after oral administration. The Δ^9-THC area under the plasma concentration versus time curve ($AUC_{(0-\infty)}$) for THC-HS was 2.44-fold higher (90% confidence interval: 1.78, 3.35) than for the capsule administration.

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E-Mail karger@karger.com
www.karger.com/mca

Mahmoud A. ElSohly
ElSohly Laboratories, Inc.
5 Industrial Park Dr.
Oxford, MS 38655 (USA)
E-Mail elsohly@elsohly.com

Introduction

Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the primary active ingredient of the plant *Cannabis sativa* L. (marijuana) and is responsible for the majority of the pharmacological effects of marijuana smoking. Marijuana has long been advocated for its medicinal value, and this is largely attributable to the activity of Δ^9-THC. During cannabis smoking, rapid absorption of Δ^9-THC occurs with smoking. Only seconds after the first puff from a Cannabis cigarette, Δ^9-THC is detectable in plasma [1], with C_{max} occurring 3–10 min after onset of smoking [2–6]. This accounts for the rapid euphoric effects of marijuana smoking or vapor inhalation, and experienced users will typically "titrate" the smoking rate to achieve the desired effect. The delivery of Δ^9-THC by other routes is delayed in onset and reduced in extent. However, in the context of therapeutics, smoking is a poor choice for delivery of Δ^9-THC, as variability in consistency and quality of the botanical raw material and different smoking practices make it difficult to dose consistently. In addition, abuse liability and impairment risk must be considered. With smoking marijuana, the tars produced during this combustion process may be carcinogenic and can also injure the bronchial mucosa, decrease airway conductance, and impair the antibacterial activity of alveolar macrophages [7, 8]. One of the best established therapeutic actions of Δ^9-THC is its remarkable effect on chemotherapy-induced nausea and vomiting. The endocannabinoid system, which mediates many of the pharmacological actions of Δ^9-THC, is now known to be an important pathway in regulation of appetite and emesis [9–15]. Marinol® (orally administered dronabinol, the pharmaceutical name for Δ^9-THC) was approved in the US in 1985 for the stimulation of appetite in AIDS-related anorexia and for chemotherapy-induced nausea and vomiting.

The appetite-stimulating effect of dronabinol for the treatment of AIDS-related anorexia was investigated in a randomized, double-blind, placebo-controlled study involving 139 patients [16]. As compared to placebo, dronabinol treatment resulted in a statistically significant improvement in appetite at 4 weeks. The study showed a sustained improvement in appetite for a full 12 months, without the need to increase the dose [17]. Dronabinol treatment of chemotherapy-induced nausea and vomiting was evaluated in 454 patients with cancer, who received a total of 750 courses of treatment for various malignancies [18].

Many other disease indications are also being explored for potential therapeutic benefits with Δ^9-THC, including pain relief, spasticity in multiple sclerosis and spinal injury, posttraumatic stress disorder, and other neurological disorders [19–21].

Thus, these studies support the effectiveness of Δ^9-THC for a number of indications. However, dronabinol oral absorption is slow and erratic, and the "first-pass" metabolic effect is high, so that when administered orally, there is a variable peak in Δ^9-THC in plasma ranging from 45 min to a few hours, and an overall systemic bioavailability of about 10% [2, 22]. In addition to these pharmacokinetic considerations, the use of capsules in many of these indications can be limited by nausea, vomiting, or difficulty in swallowing. There are a number of alternatives for systemic delivery of Δ^9-THC, including suppository administration. Studies in the first author's laboratory (M.A.E.) over a number of years have established the feasibility of this approach. Since Δ^9-THC is not absorbed rectally [2], prodrug formulations have been evaluated and, in animal studies, THC-hemisuccinate (THC-HS) was found to afford excellent potential for sustained delivery with good bioavailability and reduced "first-pass" metabolism [23]. The HS ester is absorbed across the rectal mucosa, but then hydrolyzes rapidly in plasma, releasing the active drug Δ^9-THC. The bioavailability of Δ^9-THC from THC-HS formulation in a Witepsol H15 base was demonstrated in monkeys [24]. A pilot study in 2 human subjects also suggested an improved systemic delivery of Δ^9-THC, with relatively reduced first-pass metabolism, compared to oral administration [25].

The purpose of the present study is to more fully assess the tolerability and pharmacokinetics of ascending single doses of the THC-HS prodrug via suppository formulation in healthy human volunteers, and to compare the systemic delivery of Δ^9-THC and its metabolism after oral administration of equivalent amounts as dronabinol capsules and via the prodrug in suppositories.

Methods

Analytical Methods

Materials

Δ^9-THC (1 mg/mL solution in methanol; MeOH), 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH, 0.05 mg/mL solution in MeOH), and d_3-Δ^9-tetrahydrocannabinol (d_3-THC, 1 mg/mL solution in MeOH) were purchased from Sigma-Aldrich. 11-OH-Δ^9-tetrahydrocannabinol (11-OH-THC, 0.1 mg/mL solution in MeOH) and d_6-11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (d_6-THC-COOH, 0.1 mg/mL solution in MeOH) were obtained from ElSohly Laboratories, Inc., Oxford, MS, USA. THC-HS (Batch No. NJG097/B) was obtained from MacFarlan

Smith Limited (Edinburgh, UK) and stored at 4 °C under nitrogen in the dark. THC-HS as suppositories and matching placebo suppositories containing the Wecobee M suppository base were supplied by Oxford Natural Products plc (Charlbury, UK).

Control human plasma samples (with lithium heparin anticoagulant) were available in house at Charles River-Inveresk. Acetonitrile was purchased from BDH Chemicals, MeOH from Rathburn (HPLC grade), water from Milli-Q, C_{18} SPE cartridges (100 mg/mL) from Isolute, and ammonium acetate, acetone, ammonium solution (SG 0.88), and formic acid were obtained from Fisher Scientific, UK (certified grade).

All the reagents were given a nominal 1-year expiry date from receipt at the laboratory. Milli-Q water was always prepared fresh each time of use.

Method Development

Initial development of the analytical methods showed that Δ^9-THC and its 2 main metabolites are quite stable in human plasma, with no degradation over 90 min, even at room temperature. However, this was not the case for the HS ester. THC-HS was rapidly and extensively hydrolyzed to Δ^9-THC, presumably by plasma esterases, and though this was slower at 4 °C, there was still substantial and variable hydrolysis. Therefore, assessment of pharmacokinetics of the THC-HS prodrug was not attempted.

Preparation of Calibration and Quality Control Samples

A 0.4-mL aliquot of the Δ^9-THC stock solution (1 mg/mL in MeOH) was diluted to 20 mL in a volumetric flask with MeOH/H_2O (50:50, v/v) to give solution A. To 1 mL of this solution 0.4 mL of the THC-COOH stock solution (0.05 mg/mL in MeOH) and 0.2 mL of the 11-OH-THC stock solution (0.1 mg/mL) were added, mixed in a volumetric flask, and diluted to 20 mL with MeOH/H_2O (50:50, v/v) to give solution B.

From solution B, further dilutions were made in MeOH/H_2O (50:50, v/v) to give a range of concentrations (0.005–0.8 μg/mL). A 20-μL aliquot of these solutions was spiked to 400 μL matrix to give the final concentrations of the test items. Quality control samples were prepared in the same manner as solution B. All the samples were stored at 4 °C.

Extraction Procedure

Plasma sample (400 μL) was transferred to a test tube, spiked with 20 uL (d_3-THC and d_6-THC-COOH) internal standard solutions (20 ng) and 20 μL of MeOH/H_2O (50:50 v/v). Then, 500 μL of 0.5% v/v ammonia solution (S.G. 88, saturated solution) was added, vortexed, and left for 3 h at room temperature for hydrolysis. Samples were extracted using C_{18} SPE cartridges (100 mg/1 mL) which were conditioned with 1 mL of MeOH followed by 1 mL of 0.5% SG 0.88 ammonia solution. Samples were then added to the conditioned C_{18} SPE cartridges and washed with 0.5 mL 1% formic acid solution, dried under vacuum, and eluted with 3 × 0.5 mL acetone. The acetone extract was evaporated at 40 °C and the residue reconstituted by adding acetonitrile/0.02% formic acid solution (50:50, v/v, 150 μL). The mixture was then vortexed, sonicated for 10 min, and transferred to an autosampler vial followed by centrifugation (14,000 rpm, 3 min) prior to analysis.

Instrumentation and Chromatographic Conditions

The system was comprised of a Perkin-Elmer Series 200 HPLC pump and autosampler, interfaced with Applied Biosystems Sciex AP1365 mass spectrometer. All acquisitions were performed under positive ionization mode with an ion spray voltage of +4,800 V. Nitrogen was used as the nebulizer gas as well as the drying gas with a turbo temperature of 500 °C. Ions monitored for Δ^9-THC were m/z 315.3/193.0, for d_3-THC m/z 318.3/196.2, for 11-OH-THC m/z 331.4/193.0, for THC-COOH m/z 345.4/193.0, and for d_6-THC-COOH m/z 351.4/193.0. Separation was achieved on an Inertsil ODS-3 column (50 × 2.1 mm; 3-μm particle size) equipped with a filter disc guard column. The mobile phase consisted of a mixture of acetonitrile/0.02% formic acid (50:50, v/v) (A) and acetonitrile/0.05% formic acid (80:20, v/v) (B). At a flow rate of 0.2 mL/min, a gradient elution was used as follows: 100% A from 0 to 3 min, then at 3.2 min 100% B till 13.0 min. Each run was followed by a 2.8-min wash with 100% A. Injection volumes were 10–30 μL with 0 split ratio.

Human Subjects

The study was carried out in 2 cohorts of subjects at Inveresk Research, Edinburgh, UK. In Cohort 1, ascending doses of THC-HS (5 doses ranging from 1.25 to 20 mg THC equivalent) were administered via suppository to healthy volunteers, and tolerability and pharmacokinetics were assessed over a 24-h period. This was a double-blind, randomized, placebo-controlled study, with 2 placebo subjects included at each dose level. In Cohort 2, subjects received, in open-label, randomized crossover fashion, 10 mg Δ^9-THC as Marinol® (dronabinol) capsules orally and THC-HS (containing 10 mg Δ^9-THC) suppository rectally, with a 14-day washout between the 2 treatments. Protocols were approved by the Independent Ethics Committee of Inveresk and studies were conducted according to Good Clinical Practice.

For Cohort 1, 30 male subjects aged between 18 and 50 years were recruited from the Inveresk Research volunteer database. For Cohort 2, 12 additional subjects from the same pool were recruited. All subjects weighed between 50 and 100 kg and were within ±15% of ideal body weight, with no clinically relevant abnormal physical or laboratory findings at screening, and with normal blood pressure and heart rate. All subjects consumed alcohol except one. Subjects refrained from drinking alcohol or grape fruit juice or consuming caffeine-containing products for 48 h before each admission to the clinical unit. No medications (other than study drug) were permitted during the study except paracetamol or other drugs to treat minor conditions. With respect to this, 1 subject reported a single dose of a paracetamol-based pain killer for headache and a single dose of vitamins in the 2 weeks before the start of the study. Another subject reported 1 dose of an over-the-counter preparation for cold symptoms 8 days after dosing.

Pre-Study Screening

Subjects underwent screening procedures within 14 days of dosing. The screening examination consisted of a medical history (including a record of past cannabis use), complete physical examination and vital signs, 12-lead ECG recording, hematology, clinical chemistry and urinalysis, screening for HIV, hepatitis B and C, and urine screening for drugs of abuse.

Study Drugs

THC-HS suppositories contained 1.25, 2.5, 5, 10, or 20 mg equivalents of Δ^9-THC as the HS. These were manufactured by Penn Pharmaceutical Laboratories, Gwent, UK, and formulated in Wecobee M suppository base. Placebo suppositories of Wecobee

M base were also employed. The comparator drug used in Cohort 2 was Marinol® 10 mg capsules (batch 11031098A).

Treatment Administration and Duration

Each subject was dosed with a single rectal dose of THC-HS or placebo in the morning of day 1. The dose was administered to the subject lying in the left lateral position with legs flexed at the hip and knee. The product was administered into the lower rectum and a minimum amount of lubrication was used only if necessary to achieve insertion. Subjects lay in the supine position for at least 2 h after drug administration, remained in the clinic for 24 h after dosing, and returned for a poststudy visit on day 7. For Cohort 1, the doses selected for this study were equivalent to the following doses of Δ^9-THC: 1.25, 2.5, 5, 10, and 20 mg. Four subjects received the drug and 2 received placebo at each of the 5 dose levels. These doses cover the range of doses anticipated for use in patients. For Cohort 2, the same procedure was followed with the suppository administration at a dose of 10-mg equivalent of Δ^9-THC. These subjects also received, in randomized crossover fashion, a 10 mg capsule of Marinol® with 200 mL of water. There was a 14-day washout between the 2 treatments.

Hematology/Clinical Chemistry

Laboratory tests were performed at screening, predose (0 h), and 24 h after each dose. Blood samples for clinical chemistry and hematology were collected in preheparinized tubes (4.0 mL) and EDTA-coated tubes (3.0 mL), respectively. Blood samples for clinical chemistry tests were centrifuged at 3,000 rpm for 10 min at approximately 4 °C. The separated plasma was stored at −20 °C until analyzed. Blood samples for hematology tests were refrigerated at approximately 4 °C until analysis. Laboratory tests showing abnormal values for any subject were repeated as often as was deemed necessary.

Safety Measures

Urinalysis was performed at screening, predose, and 24 h after each dose. A urine drug of abuse screen was performed at screening and on each admission to the clinical unit. A 12-lead ECG was obtained at screening, predose, and at 1, 4, 8, and 24 h after each dose. Rectal mucosa was examined by proctoscopy and was performed predose and at 4 and 24 h after administration of the suppository. A physical examination was performed at screening and at completion (day 7 after study visit). Supine systolic and diastolic arterial pressure and supine heart rate were determined by an automated recorder. The methods employed for recording vital signs were in accordance with standard operating procedures. Measurements were made at screening, on each admission, predose, and at 15 min, 30 min, 1, 2, 4, 8, 12, and 24 h after each dose.

Pharmacokinetic Analysis

For the purposes of pharmacokinetic parameter estimation, reported plasma concentrations of Δ^9-THC were taken to represent circulating concentrations of Δ^9-THC alone. THC-HS in human whole blood is very unstable, and circulating concentrations of THC-HS following rectal dosing are therefore likely to be negligible. Subsequent to import of plasma concentration data into WinNonlin, some data imputations for replacement of missing values could have been performed at the discretion of the pharmacokineticist, according to standard practice. For pharmacokinetic purposes, the predose sampling time points where concentrations were reported as "NQ" (i.e., not quantifiable) were ignored. In the absence of concentration data at time zero, WinNonlin automatically imputes a concentration value of zero when an extravascular dose model is used. Where concentrations were reported as below the limit of quantification (LOQ) ("'NQ") but samples at both previous and subsequent time points contained quantifiable concentrations, 0.5× LOQ was imputed. In contrast, where no samples at subsequent time points contained quantifiable concentrations, these were ignored for pharmacokinetic purposes.

Method of Parameter Estimation

Individual plasma concentrations of each of the 3 analytes Δ^9-THC, THC-COOH, and 11-OH-THC against actual sampling time after dosing were generated for each subject. Pharmacokinetic parameter estimates for all 3 analytes were derived for each individual before the calculation of mean parameter estimates for each analyte and dose level. Pharmacokinetic parameters were estimated using WinNonlin pharmacokinetic software (version 1.1, Pharsight Corp., Mountain View, CA, USA). A noncompartmental approach was used to generate parameter estimates, using WinNonlin model 200 (extravascular dosing). The terminal elimination phase was identified by regression analysis within WinNonlin, using at least 3 data points in each plasma concentration versus time profile.

Parameter Definitions

The following parameter estimates were calculated where possible for each subject and each analyte unless otherwise specified:

$C_{max\ (obs)}$ was determined by direct inspection of the plasma drug concentration versus time data point values. $T_{max\ (obs)}$ was also determined by direct inspection of the plasma drug concentration versus time data point values. $AUC_{(0-\infty)}$ was the area under the plasma drug concentration versus time curve, calculated by extrapolation of the elimination slope from the last plasma concentration to infinity, thus: $AUC_{(0-\infty)} = AUC_{(0-t)} + (Ct/Kel)$, Ct being the plasma drug concentration at time "t," and Kel the elimination rate constant for the drug, determined from the terminal elimination slope. The terminal elimination half-life ($T_{½el}$) was calculated by regression analysis of the terminal elimination slope.

Results

Analytical Method Development

Calibration curves for all standard compounds were constructed in the range of 0.25–25 ng/mL. The system limit of detection (LOD) was determined by analyzing standard solutions of known amounts of the 3 analytes and their deuterated internal standards. Standards were prepared with decreasing concentrations of the 3 analytes and a fixed concentration of their internal standards. The system LOD was defined as the injected amount of the 3 analytes with discernible peaks at least 3 times greater in height than the background noise. The LOD and LOQ were 0.25 and 0.50 ng/mL, respectively, for all 3 analytes. The acceptance criterion for assay accuracy was 100 ±

Table 1. Assay linearity of Δ^9-THC in human plasma

Actual concentration, ng/mL	Response ratio	Determined concentration, ng/mL	Difference, %
0.5011	0.0570	0.524	4.6
1.002	0.101	0.945	–5.7
2.505	0.266	2.53	1.0
5.011	0.518	4.95	–1.2
10.02	1.06	10.1	0.8
15.03	1.59	15.2	1.1
25.05	2.59	24.8	–1.0

Table 2. Assay linearity of 11-OH-THC in human plasma

Actual concentration, ng/mL	Response ratio	Determined concentration, ng/mL	Difference, %
0.5000	0.101	0.413	–17.4
1.000	0.198	1.07	7.0
2.500	0.420	2.58	3.2
10.00	1.69	11.2	12.0
15.00	2.26	15.1	0.7
25.00	3.53	23.7	–5.2

Table 3. Assay linearity of THC-COOH in human plasma

Actual concentration, ng/mL	Response ratio	Determined concentration, ng/mL	Difference, %
0.5010	0.175	0.419	–16.4
1.002	0.292	0.922	–8.0
2.505	0.719	2.75	9.8
5.01	1.40	5.67	13.2
10.02	2.60	10.8	7.8
15.03	3.55	14.9	–0.9
25.05	5.61	23.7	–5.4

20%, and the acceptance criterion for the assay precision was ≤20% for all 3 analytes. Their assay LOQ in human plasma was 0.5 ng/mL. The assay accuracy at this concentration was 89.8, 93.4, and 95% and the assay precision was 3.8, 16.8, and 6.6% for Δ^9-THC, 11-OH-THC, and THC-COOH, respectively.

Assay precision and linearity were assessed by 9-point calibration curves (0.025, 0.05, 0.1, 0.25, 0.5, 1.0, 1.5, 2.5, and 5.0 ng/mL) for all 3 analytes. The intra- and inter-day precision and accuracy values were determined by spiking replicate (n = 6) samples of matrix (400 µL) with standard solutions of the 3 analytes to give concentrations of 1, 8, and 20 ng/mL. A separate calibration series of matrix samples was prepared, spiked with the 3 analytes over the range of 0.5–25 ng/mL, as for the determination of assay linearity. The samples were extracted and analyzed according to the extraction procedure described above. The intra-day precision (coefficient of variation, CV) for the 1.0 ng/mL samples (n = 6) was 7.4, 2.9, and 9.5% for Δ^9-THC, 11-OH-THC, and THC-COOH, respectively. The inter-day precision (CV) for the 1.0 ng/mL samples (n = 6) was 7.2, 13.7, and 8.3% for Δ^9-THC, 11-OH-THC, and THC-COOH, respectively.

The recovery of the 3 analytes was determined by spiking aliquots of matrix (400 µL) with their standard solutions to give a range of concentrations over the linear range of assay (0.5–25 ng/mL). The samples were extracted and analyzed. A nonextracted series of standards was also prepared over the equivalent assay linear range by spiking standard solutions of the 3 analytes (20 µL) and their internal standard (20 µL) into the reconstitution solvent (110 µL). These samples were analyzed alongside the extracted samples. For each test item, a calibration curve was constructed by plotting the nonextracted test item:internal response ratio against the equivalent nominal matrix concentration of Δ^9-THC, 11-OH-THC, and THC-COOH. The criterion for acceptance was that the determined equivalent matrix concentration for each standard, as calculated from the calibration curve, was within ±15% of the equivalent nominal matrix concentration (±20% at the lower limit of linearity). The mean recoveries of Δ^9-THC, 11-OH-THC, and THC-COOH from human plasma were demonstrated to be 103.1, 83.8, and 85.8%, respectively. The mean recoveries of their internal standards from human plasma were demonstrated to be 98.5, 79.3, and 78.0%.

Assay specificity and linearity assessment showed – in matrix samples with and without the addition of the 3 analytes and their deuterated internal standards – that there were no significant interfering substances at the retention time of the test items or internal standards in human plasma. The assay was therefore deemed to be specific. A plot of the test item:internal standard response ratio against human plasma concentration of the 3 analytes demonstrated good linearity over the range of 0.5–25 ng/mL with a 1/x weighting (Tables 1–3).

The assay LOD was determined to be 0.25 ng/mL for all 3 analytes in human plasma. At this level their peaks were at least 3 times the background noise. Their assay LOQ was

Table 4. Assay limits of quantification of Δ^9-THC, 11-OH-THC, and THC-COOH in human plasma (ng/mL)

Analyte	Target concentration	Found concentration (mean ± SD)	CV	Accuracy	Precision
Δ^9-THC	0.25	0.284±0.0506	17.8	99.9665	82.2
	0.50	0.552±0.021	3.8	99.9491	96.2
	1.00	1.09±0.0414	3.8	99.912	96.2
11-OH-THC	0.25	0.285±0.126	44.2	99.965	55.8
	0.50	0.533±0.090	16.8	99.967	83.2
	1.00	1.12±0.146	13.1	99.88	86.9
THC-COOH	0.25	0.283±0.0456	16.1	99.968	83.9
	0.50	0.526±0.0347	6.6	99.975	93.4
	1.00	1.04±0.077	7.4	99.962	92.6

CV, coefficient of variation.

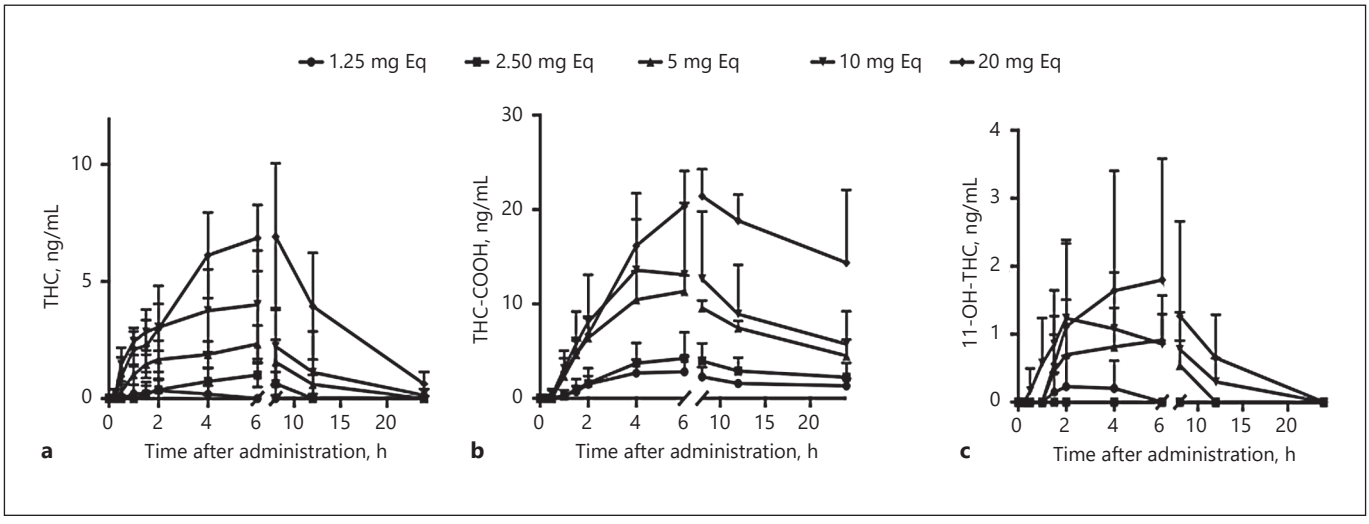

Fig. 1. Pharmacokinetic profiles of Δ^9-THC (**a**) and its major metabolites, THC-COOH (**b**) and 11-OH-THC (**c**), after suppository administration of increasing single doses of THC-HS in human volunteers. Doses shown are in terms of THC equivalents. $n = 4$ subjects at each dose.

shown to be acceptable at 0.5 ng/mL in human plasma (Table 4). At this concentration, the assay accuracy for Δ^9-THC was 89.8% (acceptance criterion: 100 ± 20%), and the assay precision was 96.2% (acceptance criterion: ≤20%). The assay accuracy for 11-OH-THC was 93.4% (acceptance criterion: 100 ± 20%), and the assay precision was 83.2% (acceptance criterion: ≤20%). The assay accuracy for THC-COOH was 95% (acceptance criterion: 100 ± 20%), and the assay precision was 93.4% (acceptance criterion: ≤20%). All the samples were run in replicates of 6.

Pharmacokinetic Findings

Cohort 1

The concentrations of the 3 analytes (Δ^9-THC, Δ^9-THC-COOH, and 11-OH-THC) for all subjects after ascending single doses of THC-HS are shown in Figure 1. At the lower dose levels of 1.25 and 2.5 mg THC equivalents, most of the analytes were near or below the quantifiable limit of 0.5 ng/mL plasma. At a higher dose of 5 mg equivalent and up, both Δ^9-THC and 11-OH-THC were quantifiable until at least 6 h after dosing. THC-COOH

Table 5. Pharmacokinetic parameters for Δ^9-THC, THC-COOH, and 11-OH-THC with increasing doses of THC-HS by suppository administration

THC-HS	Geometric mean (CV%)					
	$C_{max\ (obs)}$, ng/mL			$AUC_{(0-\infty)}$, ng × h/mL		
	Δ^9-THC	THC-COOH	11-OH-THC	Δ^9-THC	THC-COOH	11-OH-THC
1.25 mg Eq Δ^9-THC	1.013 (45.9)	3.078 (37.1)	11.180 (n/a)	–	55.517 (43.3)	–
2.50 mg Eq Δ^9-THC	0.953 (50.3)	4.414 (51.8)	20.086 (27.9)	–	180.300 (n/a)	–
5 mg Eq Δ^9-THC	2.431 (29.6)	12.602 (12.5)	42.620 (n/a)	20.318 (30.2)	277.229 (43.4)	11.180 (n/a)
10 mg Eq Δ^9-THC	3.977 (53.3)	10.594 (130.1)	1.641 (54.8)	33.621 (120.8)	266.802 (161.0)	20.086 (27.9)
20 mg Eq Δ^9-THC	7.666 (28.7)	22.463 (8.6)	2.087 (68.6)	79.954 (33.3)	459.283 (35.0)	42.620 (n/a)

Geometric means and coefficients of variation (CV) are shown for the maximum plasma concentration and the total exposure at each dose. n/a, not applicable.

was quantifiable until at least 12 h after dosing across the full dose range used in the study. For all 3 analytes, parameters were generally consistent with extravascular (oral or rectal) dosing. In most of the profiles, there was a short lag phase between 0.5 and 2.0 h before quantifiable analyte was apparent. Thereafter, a steady increase in plasma concentrations of all 3 analytes was observed. In some Δ^9-THC profiles, multiple concentration maxima were evident generally between 0.5 and 12 h after dosing. Plasma concentrations did not start to decline, following THC-HS rectal dosing, until 6–8 h after dosing.

On rectal administration of THC-HS, systematic exposure of Δ^9-THC increased with increasing the dose (Fig. 1a). This trend was evident during the full dose range, although $C_{max\ (obs)}$ slightly decreased as the dose increased from 1.25 to 2.5 mg equivalents. And no mean slope-dependent parameter estimates ($AUC_{(0-\infty)}$), $t_{½el}$, and CL/F were available for these 2 lowest dose groups because lambda-Z could not be determined. As can be seen in Table 5, the increase in the mean $AUC_{(0-\infty)}$ estimates is dose proportional over the dose range of 5–20 mg Eq THC. $T_{max\ (obs)}$ estimates exhibited no consistent trends with increasing dose. Median estimates for the 1.25, 2.50. 5, 10, and 20 mg equivalent THC doses were 4, 6, 5.01, 3.01, and 7.04 h, respectively. Reliable estimates of $T_{½el}$ were available for 5, 10, and 20 mg THC equivalent doses only, but were broadly similar (3.79, 3.76, and 4.52 h, respectively). Clearance (CL/F) estimates reflected the observed dose proportionality in $AUC_{(0-\infty)}$ estimates in the dose range of 5–20 mg THC equivalent, with no consistent trend and also variable, with mean estimates for 5, 10, and 20 mg THC equivalent doses of 253.13 L/h, 400 L/h, and 258.43 L/h, respectively.

For THC-COOH, plasma concentrations were higher and more sustained compared to Δ^9-THC (Fig. 1b), with much higher AUCs (Table 5). Mean $AUC_{(0-\infty)}$ estimates increased dose proportionally at the low doses, but at 10 and 20 mgEq THC, the dose proportionality appeared to decline. $T_{max\ (obs)}$ estimates were broadly similar across all the doses. Median estimates for the 1.25, 2.50, 5, 10, and 20 mg Eq THC doses were 7.00, 7.00, 6.00, 5.00, and 7.01 h, respectively. There was no effect of increasing dose on $T_{½el}$. Mean estimates were 13.27, 17.32, 16.88, 16.64, and 11.44 h for the 1.25, 2.50, 5.0, 10.0, and 20.0 mg Eq THC doses, respectively.

For 11-OH THC, concentrations were generally very low, but highly variable (Fig. 1c). Estimates indicative of systematic exposure to $AUC_{(0-\infty)}$ and $C_{max\ (obs)}$ increased with increasing dose (Table 5). However, for the lower THC doses, no parameter estimates were calculable due to lack of quantifiable concentration data. For $AUC_{(0-\infty)}$, with dose increase at a ratio of 1:2:4 (i.e., from 5 to 20 mg Eq THC), mean estimates also increased at a ratio of 1:1.8:3.8.

Cohort 2

The concentrations of the 3 analytes (Δ^9-THC, Δ^9-THC-COOH, and 11-OH-THC) in 12 subjects receiving, in crossover fashion, oral dronabinol 10 mg capsules and THC-HS (10 mg Δ^9-THC equivalents) are shown in Figure 2. T_{max} was significantly later and $C_{max\ (obs)}$ was lower for all analytes after rectal administration of THC-HS, but systemic exposure to Δ^9-THC (as indicated by estimates of $AUC_{(0-\infty)}$) was higher compared to oral dosing (Table 6). The ratio of $C_{max\ (obs)}$ for THC-HS versus oral dronabinol was 0.713 (90% CI: 0.562, 0.904), and the ratio of

Table 6. Pharmacokinetic parameters for Δ^9-THC, THC-COOH, and 11-OH-THC with increasing doses of THC-HS by suppository administration

Treatment	Geometric mean (CV%)					
	$C_{max\ (obs)}$, ng/mL			$AUC_{(0-\infty)}$, ng × h/mL		
	Δ^9-THC	THC-COOH	11-OH-THC	Δ^9-THC	THC-COOH	11-OH-THC
Dronabinol 10 mg oral	9.21 (43.9)	55.00 (21.1)	5.10 (52.7)	19.59 (51.2)	682.05 (47.2)	25.59 (39.5)
THC-HS suppository (10 mg THC equivalent)	6.57 (25.7)	20.50 (8.1)	2.23 (78.7)	47.84 (25.3)	655.75 (57.1)	29.91 (73.3)

Geometric means and coefficients of variation (CV) are shown for the maximum plasma concentration and the total exposure at each dose. The ratios of $C_{max\ (obs)}$ and $AUC_{(0-\infty)}$ for Δ^9-THC were significantly different for the 2 treatments (see text).

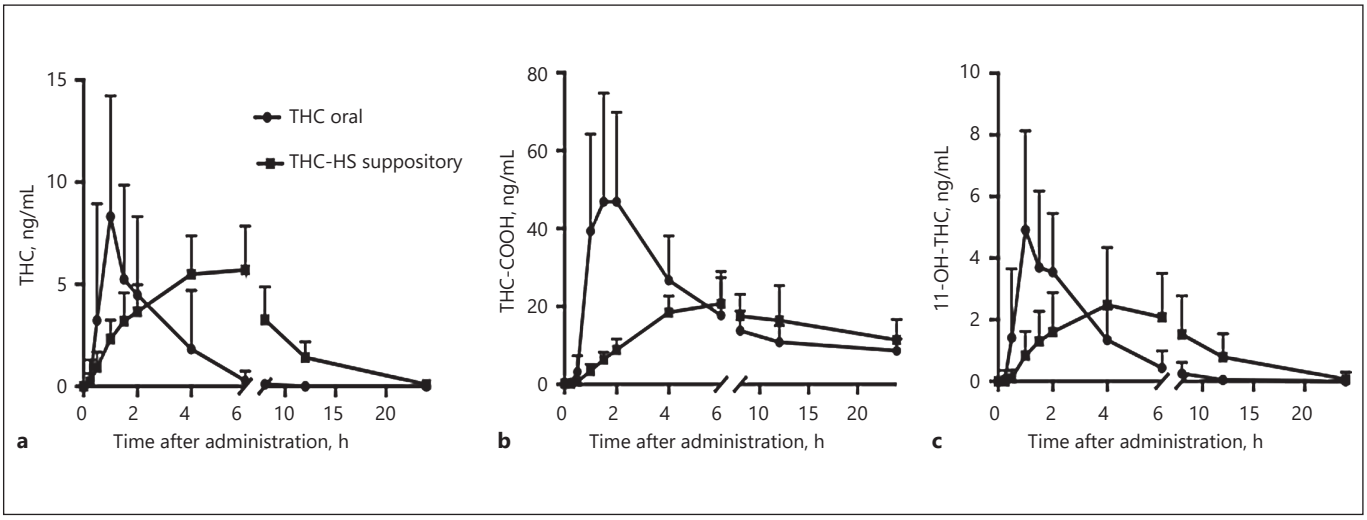

Fig. 2. Pharmacokinetic profiles in 12 human volunteers of Δ^9-THC (**a**) and its major metabolites (**b**, **c**) comparing, in crossover design, the oral administration of 10 mg dronabinol (Δ^9-THC) capsules with the rectal administration of THC-HS (10 mg Eq of THC).

$AUC_{(0-\infty)}$) for THC-HS versus oral dronabinol was 2.443 (90% CI: 1.779, 3.354). These confidence intervals for the ratio do not include 1, indicating that the parameters are significantly different. For the 2 metabolites, $C_{max\ (obs)}$ and $AUC_{(0-\infty)}$ ratios were not significantly different between oral and rectal administration.

Discussion

An analytical method was developed and validated for the determination of Δ^9-THC, 11-OH-THC, and THC-COOH in human plasma when profiling pharmacokinetics of oral Δ9-THC and rectal Δ^9-THC-HS. It was observed during the development of the analytical method that THC-HS was not stable in plasma. Thus, any THC-HS present in clinical samples would hydrolyze to Δ^9-THC, but the extent of this hydrolysis would vary from sample to sample, depending on the time taken to prepare the plasma sample from the whole blood and the time taken to process the plasma sample for analysis. The measured Δ^9-THC concentrations would be a combination of Δ^9-THC concentrations in the blood at the time the sample was collected and the concentration of the Δ^9-THC formed from the hydrolysis of THC-HS present in the sample. The analytical method was therefore employed by ensuring that any residual THC-HS would be completely hydrolyzed to Δ^9-THC and that the Δ^9-THC mea-

sured would be total Δ^9-THC (THC plus Δ^9-THC derived from THC-HS). In cases where the administered drug was Δ^9-THC, the determined total Δ^9-THC concentration using this analytical method would, in fact, be the Δ^9-THC concentration.

The intra- and inter-day accuracy (defined as the mean percentage determined concentration/nominal concentration) of the method for all the analytes at each concentration was found to be acceptable and met the criteria set out in the protocol of being within 100 ± 15%.

The intra- and inter-day precision (defined as the coefficient of variation of the mean determined concentration) of the method at each concentration was found to be acceptable and met the criteria set out in the protocol of being ≤15%. The method is therefore deemed suitable for the determination of Δ^9-THC, 11-OH-THC, and THC-COOH in human plasma.

The current study demonstrates that the delivery of Δ^9-THC via a prodrug suppository formulation of the HS ester is well-tolerated in human volunteers, and supports a strategy to enhance systemic bioavailability and increase the duration of therapeutic plasma concentrations. Earlier animal studies [23, 24] from our laboratory and a report from our collaborators on 2 human cases [25] suggested the potential utility of this approach. After oral administration, systemic bioavailability of Δ^9-THC is sharply limited and variable because of delayed and erratic absorption and a prominent "first-pass" metabolism in the liver [22, 26]. By employing the prodrug delivery of Δ^9-THC via rectal administration of THC-HS in suppository formulation, it is hypothesized that it is possible to avoid potential acid lability of Δ^9-THC in the stomach [27], and the direct exposure via the portal circulation to the dominant hepatic extraction of Δ^9-THC [28].

Such an interpretation appears to be supported by the current results. First of all, Δ^9-THC exposure rose in proportion to dose after administration of escalating doses of THC-HS by suppository, likely reflecting a slower absorption, reduced metabolism, and rapid release of Δ^9-THC in plasma upon hydrolysis of the HS ester. More importantly, in the cohort directly comparing the administration of dronabinol orally with the rectal administration of the THC-HS suppository in a crossover fashion, the exposure to Δ^9-THC was enhanced by a factor of 2.4, even though maximum plasma concentrations were slightly reduced. In addition, the exposure to THC-COOH, the major plasma metabolite, was not increased with the suppository administration, in spite of higher Δ^9-THC exposure. This indicates that the relative proportion of Δ^9-THC metabolized to THC-COOH is reduced.

Metabolism of Δ^9-THC in the human liver is predominantly via hydroxylation by cytochromes P450 (CYPs), the major metabolite being 11-OH-THC, formed by the action of CYP2C isoenzymes [29–31]. 11-OH-THC is a psychoactive metabolite, perhaps even more active than Δ^9-THC [32]. In the current study, it shows a similar kinetic profile to Δ^9-THC, as has been reported previously [33]. 11-OH-THC is further oxidized to THC-COOH, which is not psychoactive, but is a persistent metabolite in human plasma [1] and urine [34].

The analytical method developed here for Δ^9-THC and the two main metabolites in human plasma was validated extensively and is sensitive, specific, and robust. These will find utility in therapeutic monitoring of Δ^9-THC delivered by various routes.

Delivery of Δ^9-THC via the suppository route could have several practical advantages. In disease indications where emesis (as in chemotherapy-induced nausea and vomiting) or swallowing difficulty (e.g., multiple sclerosis) is present, rectal administration could be preferable. The longer and higher exposure (relative to oral dosing) could be convenient for therapeutic coverage (e.g., allowing twice a day dosing). In addition, the slower rate of rise in Δ^9-THC concentrations may minimize psychoactive effects, which may reduce some side effects.

In this study, the suppository formulations were well tolerated, and no safety issues were observed. Clearly, studies with subchronic administration for safety and efficacy in various indications are important, but these findings and the preliminary reports of positive benefit in spasticity due to spinal injury [27] suggest an excellent alternative approach to delivery of Δ^9-THC for these and other indications.

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Author Contributions

M.A.E., W.G., and L.A.W. conducted background research. M.A.E., Fiona M. Milligan, and G. M. McGuire were involved in study design and execution of pharmacokinetic studies. M.A.E. and W.G. contributed to the analytical method development and reviewed the validation data. L.A.W. organized the manuscript draft. All authors contributed to writing and finalizing the text of the manuscript.

Disclosure Statement

The authors have no financial or nonfinancial competing interests. The product evaluated is no longer under any intellectual property or commercialization agreement.

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