Title: Cannabidiol and CES1 Interactions in Healthy Subjects

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College of Pharmacy, Department of Pharmacotherapy & Translational Research (PTR), College of Pharmacy

Clinical Research Protocol

An Assessment of the Drug Interaction Potential Between Oral Cannabidiol (Epidiolex®) and the CES1 Substrate Methylphenidate in Healthy Volunteers

IRB Protocol No: **202002547**

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Study Sponsor: State of Florida Consortium for Medical Marijuana Clinical Outcomes Research

PREFACE: *COVID-19 CONSIDERATIONS*

Because of the COVID-19 viral pandemic, certain precautions for the personal safety of the subjects and the safety of UF study staff and personnel must be maintained. Although it is anticipated that the specific nature of these precautions will evolve over time (and the course of the study), certain basic precautions shared by UF and Shands Faculty, staff, students and visitors will be observed that may be subject to change per UF guidelines.

Accordingly, all research subjects, will be required to wear a mask or cloth face covering when in UF and UF Health facilities. Face coverings must be worn in all patient care/ research areas and when in public/common areas, including lobbies, elevators, stairwells, and bathrooms. Further, the subject and one or more people are within 6 feet of each other outside of any UF building, they must also wear a mask or cloth face covering. This is explained explicitly in the Informed Consent document. There may be the need to show evidence of a negative COVID-19 test prior to participation in the study.

While in the CRC research unit where subject evaluations and primary study day activities will take place, there may be further directions provided by CRC staff related to CRC-specific policy and procedures regarding these precautions. Such procedures may include a temperature checks and several questions about any recent travel or known contact with COVID-19 positive individuals by the research subject. Any such CRC unit specifics are not available to the PI at the time of this submission. Nevertheless, this possibility is also explained to subjects in the Informed Consent document.

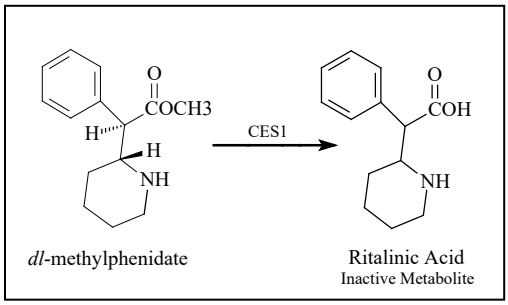
1. INTRODUCTION

Introduction and Background

Epidiolex® (cannabidiol [CBD]) solution is an FDA-approved medication derived from cannabis that is approved for the treatment of seizures associated with Lennox-Gastaut syndrome (LGS) or Dravet syndrome (DS) in patients 2 years of age and older. Additionally, CBD and CBD in combination with other cannabinoids is presently being studied for an expanding array of other pediatric clinical indications including Childhood Absence Seizures, Prader-Willi Syndrome, Autism Spectrum Disorder, Fragile X Syndrome, Attention Deficit Hyperactivity Disorder (ADHD), and others.[1] Importantly, ADHD is the most common neuropsychiatric disorder of childhood, often persists into adulthood and the psychostimulant *d,l*-methylphenidate (Ritalin®) accounts for approximately 50% of all stimulant usage in the world. In the US alone there has been an approximately 10-fold increase in methylphenidate (MPH) prescribing since 1990, with 18 million prescriptions dispensed in 2010, including 1.9 million new starts on MPH, making it the 5th most commonly prescribed medication to children ages 2 -11 and the single most frequently prescribed medication of any type in those aged 12-17 years.[2]

Thus, more likely than not, a very large number of pediatric patients who are receiving cannabidiol and/or other cannabinoids in clinical studies or in clinical care, will also be receiving MPH concomitantly. Any DDI between the two would be of clinical relevance.

One of the most important aspects of safety considered in all drug therapies is the potential for the occurrence of drug-drug interactions (DDIs) of clinical significance. In the case of Epidiolex® (cannabidiol), the product sponsor, GW / Greenwich, has performed the requisite *in vitro* and *in vivo* DDI assessments as part of the FDA drug approval process.[3] However, these studies were almost exclusively assessments of the potential influence of CBD on the Phase I cytochrome P450 (CYP) enzyme system. **Recently, our group carried out the first *in vitro* assessment of the effects of CBD and other major cannabinoids on another Phase I drug metabolizing enzyme carboxylesterase 1 (CES1)**.[4]



The results of our just published study indicate that CBD and other cannabinoids can significantly inhibit CES1. This finding is significant in that functional and unimpeded hepatic CES1 is responsible for both the *detoxification/deactivation* of numerous CES1 substrates including methylphenidate (which relies almost exclusively on the CES1 pathway and no CYP 450 enzymes). Indeed in a reaction catalyzed by CES1, MPH is exclusively hydrolyzed to the inactive metabolite ritalinic acid.[5] Additionally, CES1 is involved in the deactivation of numerous other substrates and also the *activation* of a number of medications formulated as prodrugs (e.g. oseltamivir). **Table 1** contains numerous examples of known CES1 substrates including chemicals, toxins, and medications representing essentially every major therapeutic drug class.[6]

Potential Drug-drug Interaction Implications

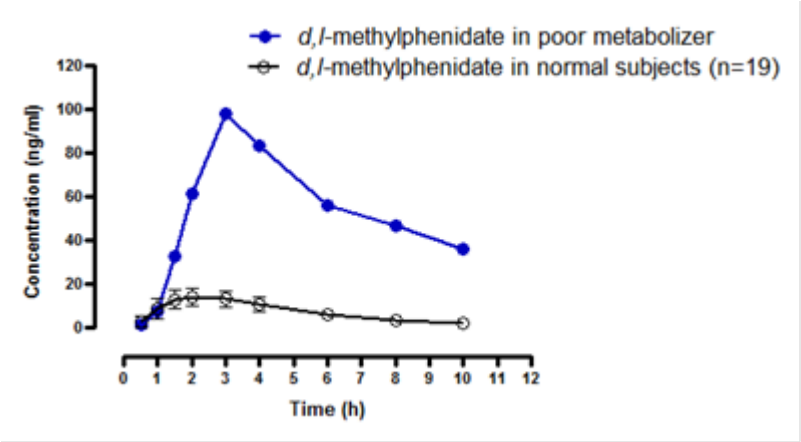
Our recently published *in vitro* studies suggest that the use of CBD alone and or in combination with other cannabinoids, concurrently with MPH would be anticipated to impede the normal deactivation (de-esterification) of MPH, and potentially lead to concentration associated adverse effects or toxicities.

However, *in vitro* studies,

ACE inhibitors		**CNS Agents**	**Antihyperlipidemics**
benazepril	perindopril	methylphenidate	simvastatin
enalapril	quinapril	cocaine	lovastatin
Imidapril	ramipril	heroin	clofibrate
moexipril	trandolapril	meperidine	fenofibrate
		flumazenil	
Antiplatelets/Anticoagulants		**Anticancer Agents**	**Chemical warfare agents**
clopidogrel		capecitabine	sarin
dabigatran etexilate		irinotecan	soman
			tabun
Antiviral Agents		**Immunosuppressive**	**Pesticides**
oseltamivir		mycophenolate mofetil	trans-permethrin
sofosbuvir		ciclesonide	para-nitrophenyl valerate
Endogenous compounds		**Miscellaneous**	**Synthetic Cannabinoids**
cholesterol		oxybutynin	AB-PINACA, AB-FUBINACA
fatty acid ethyl esters			PB-22, 5F-PB-22

Table 1: **Substrates of CES1 as established by in vitro assay**

particularly of natural products, have a number of recognized limitations and *in vitro* indications of DDI liability are not always borne out in clinical confirmatory studies.[7] Accordingly, an assessment of whether these recent *in vitro* findings translate into a clinically relevant DDI requires confirmation through a formal interaction study in healthy individuals. In 2008[8] we documented the first genetically deficient (*CES1*) metabolizer of MPH- the consequences of which are seen in the figure below (vs 19 normal



subjects). As can be appreciated from this example, impaired CES1 activity can result in drastic elevations of MPH, even after a single dose of an immediate-release formulation. Finally, beyond the concerns of CES1 inhibition which may lead to the inability adequately to deactivate/detoxify and clear a given CES1 substrate (e.g. methylphenidate) is the inability to *activate* a number of prodrugs (e.g. oseltamivir) which require functional CES1 to be converted to their active moiety.[9]

Clinical Evidence of CBD Inhibition of CES1 Activity

Gaston and coworkers[10] conducted a study which assessed the potential pharmacokinetic interactions between CBD (Epidiolex®) and commonly co-administered antiepileptic drugs (AEDs) in adults and children with treatment-resistant epilepsy. Notably, among other findings, CBD was found to dose-dependently, and significantly, increase concentrations of rufinamide (Banzel®), an AED almost exclusively metabolized via CES1. The investigators were unable to articulate a clear mechanism of the drug-drug interaction based upon the known *in vitro* activities of CBD and speculated that the sesame oil vehicle used in the Epidiolex® formulation might play a role. However, our recent findings lead us to believe that CBD inhibition of CES1, an unknown phenomenon at the time of the Gaston et al publication, is the likely explanation. The magnitude of the interaction is not well described in the paper and there are a number of study limitations that would limit such determinations. First, subjects were started on 5 mg/kg/day (in divided doses) and potentially titrated to a maximum of 50 mg/kg/day but it is not reported what final CBD doses were. Secondly, only single blood samples for rufinamide were obtained and these were drawn during a 4-hour sampling window rather than a narrow range (i.e. 1 hour) more typical of rigorous drug-drug interaction assessment. A significant CBD-

rufinamide interaction would not necessarily be an impediment to using the two together, but clinical awareness is important relative to dosing. Indeed, CBD might actually lessen the dosing requirements for rufinamide and decrease the cost of treatment.

3. PURPOSE OF PRESENT STUDY

To address the question of whether CBD can inhibit CES1 activity in a clinically relevant manner, we propose the conduct of a small (n=12) healthy volunteer study assessing the potential influence of Epidiolex® (cannabidiol) on the pharmacokinetic disposition of a single dose of the known CES1 substrate MPH in healthy volunteers. Such an assessment will directly answer the question of whether orally administered CBD, at clinically relevant doses, can significantly alter (impede) the metabolism of co-administered MPH. Furthermore, such an assessment, wherein MPH also functions as a CES1 probe drug substrate, will also provide some insight as to the possibility of CBD influencing a wide array of other CES1 substrates (**Table 1**).

4. HYPOTHESIS

Epidiolex® (CBD) will produce modest increases in the C_{max} and area under the plasma concentration-time curve from 0 to 8 hours ($AUC_{0\rightarrow 8h}$) of a single dose of the recognized CES1 substrate, *dl*-methylphenidate (Ritalin®) in healthy volunteers and confirm our recent *in vitro* findings.

5. SPECIFIC AIMS

The Specific Aims are as follows:

1) To determine if the single dose pharmacokinetics of the psychostimulant and CES1 substrate, methylphenidate (Ritalin® others), are altered by concomitant use of Epidiolex® (CBD).

2) To determine if the single dose pharmacokinetics of the psychostimulant and CES1 substrate, methylphenidate (Ritalin® others), are altered by concomitant exposure to the inactive components in the vehicle solution used for the Epidiolex® formulation

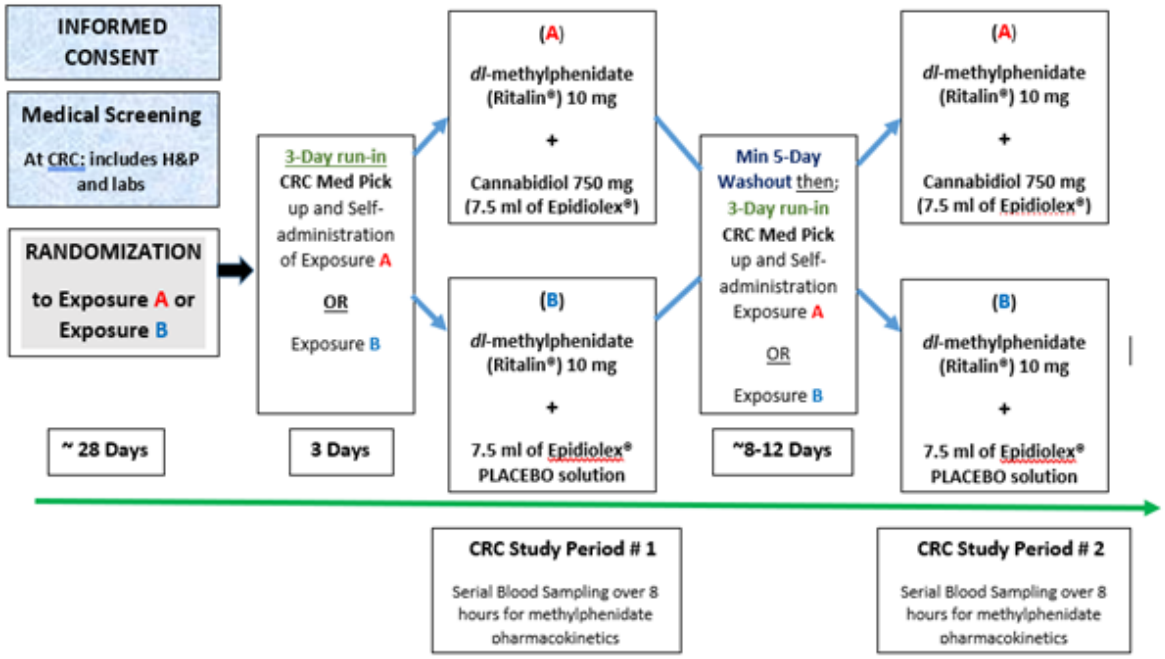
6. METHODS

A. Clinical Protocol Overview

An open-label, randomized crossover design is proposed wherein healthy volunteer subjects (n=12) would receive a single dose of immediate-release *dl*-methylphenidate (Ritalin®) concomitantly with orally administered CBD (Epidiolex®) solution or an equal volume of Epidiolex® placebo solution (i.e vehicle with no CBD) which have been dosed to plasma steady-state conditions. Serial blood samples will then be collected for methylphenidate analysis over the ensuing 8-hour period during both Epidiolex® exposure phases. Methylphenidate concentrations will be determined using a validated LC-MS/MS assay.[11] Standard pharmacokinetic parameters will be calculated including methylphenidate peak concentration

(C_{max}), the time to C_{max} (t_{max}), the terminal elimination half-life ($t_{1/2\beta}$) and the area under the plasma concentration-time curve (AUC). Comparisons between study phases would then reveal any significant influence of CBD. The study lasts about 6 weeks and involves 5 visits to the CRC although 2 of these 5 visits are only to pick up study medications (see **Table 2**)

A general schematic of the proposed clinical study design is presented in the Figure Below:



Research Subjects

The study population will consist of normal healthy volunteers. The study population will consist of 12 healthy adult subjects (6 females, 6 males completing the study), aged 21-45 years, who are healthy as assessed by medical history, physical examination, and routine laboratory tests including complete blood count, Comprehensive Metabolic Panel, urinalysis, and urine tetrahydrocannabinol (THC) screen. THC is a surrogate marker for cannabis use which would generally also result in exposure to CBD as well. Since outside use of CBD could confound the proposed study, THC positive subjects will be excluded. Participating females of child-bearing potential must have a negative urine pregnancy test prior to enrollment and avoid pregnancy during study participation. Female subjects will be tested prior to each scheduled study day (urine pregnancy test) to provide additional assurance. A subject's Body Mass Index (BMI) must be between 18.5 to 28 kg/m^2 (inclusive).

With the exception of oral contraceptives, no other prescription or OTC medications are permitted during study participation. These restrictions also extend to botanical/nutritional supplements, vitamins and "energy drinks". All subjects must have no clinically significant diseases or clinically significant abnormal laboratory values as assessed during the screening

medical history, physical exam, and laboratory evaluations. Informed Consent forms must be signed by the eligible subject prior to the initiation of any study procedures.

The specific Inclusion/Exclusion criteria are as follows:

Inclusion Criteria

* Signed Informed Consent
* Age: 21-45 years
* Gender: males and females (50:50)
* Race or ethnicity: no restrictions
* Body Mass Index (BMI) between 18.5 to 28 kg/m^2 (inclusive)
* Satisfactory completion of the screening medical history, physical exam, and laboratory evaluations.
* Females of child-bearing potential must have a negative urine pregnancy test prior to enrollment and avoid pregnancy during study participation.
* With the exception of oral contraceptives, subjects must not be taking prescription or OTC medication for the duration of study participation
* Subjects must have no ongoing use of any botanical/nutritional supplement, vitamin, or energy drink for the duration of study participation
* Negative Urine THC screen.

Exclusion Criteria

* The presence of a known allergy, hypersensitivity, or adverse reaction to CBD or cannabis, or sesame seed oil
* The presence of a known allergy, hypersensitivity, or adverse reaction to methylphenidate or dexmethylphenidate (Focalin®)
* A history (within the past year) or presence of clinically significant cardiovascular, cerebrovascular, renal, hepatic, gastrointestinal, pulmonary, immunological, hematological, endocrine, or neurologic disease will render subjects ineligible for the study.
* The presence of any surgical or medical condition (active or chronic) that may interfere with drug absorption, distribution, metabolism, or excretion including;
    - Gastric bezoar
    - Swallowing disorders
    - Strictures
    - Fistulas
    - GI obstruction
    - Severe dsyphasgia
    - Crohn's disease
    - Diverticulitis

* A positive urine pregnancy test.
* A positive Urine THC Screen
  - we will not retain any information on any subjects with a positive urine THC screen, including the reason for excluding them from the study.
* Any concomitant prescription medication, OTC medication, herbal or other dietary supplement or vitamins during the study period.

All subjects must be medication-free from 7 Days before initiation of the first active study day, through the duration of the study. This exclusion the use of vitamins, herbal preparations and OTC supplements.

Informed Consent/Screening Process:
All subjects will provide written informed consent approved by the University of Florida Institutional Review Board (IRB) prior to participating in the study. The Screening Visit will be conducted in the UF Shands CRC located in the Clinical Translational Research Building in Gainesville. It is expected to last approximately 1 hour.

Up to 18 subjects may screened for the clinical protocol with a goal of 12 subjects completing the entire protocol. All study protocols, informed consent forms, and Health Insurance Portability and Accountability Act (HIPAA) forms must be approved by the University of Florida (UF) Investigational Review Board (IRB) prior to initiating any study procedure or recruitment. At a screening/informed consent visit, the study design, purposes and inclusion/exclusion criteria will be explained to each prospective subject. Written informed consent will be obtained prior to commencing any study procedures**.**

After obtaining written Informed Consent, study subjects will be interviewed about their medical history and the protocol's Inclusion/Exclusion criteria will be discussed. All potential participants must be nonsmokers, not taking prescription or over-the-counter medications or botanical/nutritional supplements (inclusive of vitamins). Additionally, participants are requested to abstain from alcohol use 24 hours prior to the study health screen lab work or any scheduled study visit should they participate fully in the study.

During the screening visit, subjects will have blood samples drawn for health screening purposes including baseline serum chemistries including liver enzymes, complete blood count (CBC), urinalysis, a urine THC screen, and a urine pregnancy test (women) which will have a history and physical exam performed by the study physician (See Appendix for details). Copies of the laboratory results will be made available to study subjects at their request, and this is conveyed in the Informed Consent.

Study Visits

This section describes the study procedures for the two major study visits at the University CRC following Informed Consent and a satisfactory history and physical and medical screening.

Cannabidiol Pre-Exposure: In a previous normal volunteer study, subjects dosed with 750 mg of of Epidiolex® (cannabidiol) solution twice daily achieved steady-state conditions after approximately two (2) days.[13] Accordingly, three (3) days prior to their first scheduled full pharmacokinetic study day at the CRC, and depending on assigned randomization sequence (to be assigned by the UF Investigational Pharmacy Services), subjects will have commenced self-dosing with either **A**) CBD 750 mg (administered as 7.5 ml of Epidiolex® solution [100 mg/ml] of CBD) or **B**) 7.5 ml of Epidiolex® placebo solution containing no CBD.

CRC Check in and Preparation

Following an overnight fast (abstention from eating any foodstuffs after 9 pm the evening prior to the scheduled visit), subjects will arrive at the UF Clinical and Translational Science Institute CRC the morning of each of the two active Study Days where they will remain for approximately 8 hours for each of two separate formulation administration and blood drawing phases (for pharmacokinetic assessments) of the study. After checking in, and under medical supervision, skilled CRC staff will place an indwelling venous catheter in each subject's arm to facilitate serial blood sampling. Female subjects will provide a urine sample for a pregnancy test, the results of which will be read and documented. Baseline blood pressure, heart rate and temperature, will be obtained and vital signs (B.P. and H.R. only) will be repeated at approximately 12 noon, prior to lunch.

Drug Administration

At approximately 8:00 AM, on the day of the first full day dosing and pharmacokinetic assessment, subjects will be fed a standardized breakfast which they will have approximately 30 min to consume. After breakfast, subjects will be administered one (1) 10 mg tablet of *dl*-methylphenidate (Ritalin®) and depending on the previous randomization sequence, either **A**) CBD 750 mg (administered as 7.5 ml of Epidiolex® solution [100 mg/ml] of CBD) or **B**) 7.5 ml of Epidiolex® *placebo* solution containing no CBD. Subjects will then be given 240 ml of room temperature water and asked to drink it in its entirety. A standard lunch will later be provided to all subjects approximately 4 hours post-dosing. The composition and amount of food eaten will be recorded. Subjects will consume an identical meal on the subsequent second study visit.

Blood sample collection and processing for methylphenidate analysis

An indwelling venous catheter will be placed to facilitate serial blood sampling. A total of 9 blood samples (~10 ml each) will be taken over an 8-hour period during each of the two

primary study days. Blood samples will be collected in one 6 ml gray stoppered Vacutainers® (Becton Dickinson) which contain sodium oxalate to prevent clotting and sodium fluoride to prevent methylphenidate hydrolysis).
Specific time points of blood collection will be immediately prior to the dose (0 time point), of methylphenidate (Ritalin®) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 hrs. All samples will be drawn in heparinized tubes and stored on ice until centrifugation at 4°C. Each ~10 ml of whole blood sample is anticipated to yield ~ 5 ml of plasma following centrifugation. Plasma samples will be split into *duplicate*, approximately equal volumes (i.e. ~2.5 ml) labeled storage tubes, and frozen immediately at –70°C. CRC lab personnel will be asked to be split individual plasma samples and store them in duplicate as a measure of redundancy should the investigators encounter unanticipated difficulties in conducting the analysis.

This single dose assessment of methylphenidate pharmacokinetics will be conducted both in the presence and absence of CBD as a single dose assessment in a randomized crossover fashion. Following the completion of the initially randomized Epidiolex® exposure (active vs placebo) administration and sample collection, a minimum 5-day wash-out period will transpire prior to a return for the second single-day assessment of the alternate assignment (under identical study conditions and collection times). Based upon the relatively short half-lives of methylphenidate and CBD, 5 days will be an adequate wash-out period.

There will again be a 3-day self-dosing with either **A**) CBD 750 mg (administered as 7.5 ml of Epidiolex® solution [100 mg/ml] of CBD) or **B**) 7.5 ml of Epidiolex® PLACEBO solution prior to the second CRC Study Day.

The General Schedule of Study Events is depicted below in Table 2:

TABLE 2							
STUDY EVENT	**Study Consent** **Enrollment**	**History & Physical with screening labs drawn**	**Pick up "run-in" meds from CRC**	**PK Sampling Day #1**	**Wash-out 5-day min**	**Pick up "run-in" meds from CRC**	**PK Sampling Day #2**
VISIT #	**0**	**1**	**2**	**3**		**4**	**5**
Study week	**1**	**2**		**3**	**4**		**5-6**
Informed Consent	**X**						
Medical History		**X**					
Physical Exam		**X**					
Body Weight/BMI		**X**					
Height		**X**					
Vital Signs		**X**		**X**			**X**
Basic metabolic panel, LFTs, CBC, urinalysis, urine THC		**X**		**LFTs***			**LFTs***
Urine Pregnancy Test		**X**		**X**			**X**
Administer Study Drugs				**X**			**X**
Intensive PK Sampling Day (CRC)				**X**			**X**

*A second LFT (after baseline screening) will be drawn only on the PK visit in which active Epidiolex is given (dependent on randomization)

Compliance with Outpatient CBD Dosing

There is no way to absolutely confirm compliance in studies that involve self-administration of medications. However, as a measure to promote compliance, research subjects will be asked to save the empty oral syringes containing their CBD doses and bring them to the CRC at the time of check-in so that the investigators can examine and count them and confirm they were used.

Analytical Methods

Methylphenidate analysis

All analytical methods for this project have been developed, validated, and published by the PI previously.[10] This validated LC-MS/MS enantioselective method is established in the PI's laboratory will be utilized to quantify *d*- and *l*-methylphenidate plasma concentrations.

Pharmacokinetic Analysis

The enantiospecific assay will permit calculation of separate pharmacokinetic values for *d*- *l*- as well as total methylphenidate concentrations. Estimates will be made using a non-compartmental analysis using WinNonlin 5.3 (Pharsight, Mountain View, CA). The C_{max} and time to maximum plasma concentration (T_{max}) will be obtained directly from the plasma concentration-time data. The terminal elimination rate constant (λ_z) will be estimated by linear least-squares regression of the terminal portion of the plasma concentration-time curve, and the corresponding elimination half-live ($t_{1/2}$) was then calculated using the formula $t_{1/2} = 0.693/\lambda_z$. The area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0\rightarrow inf}$) for these single-dose assessments and $AUC_{0\rightarrow 8h}$ will be calculated according to the linear trapezoidal rule. The apparent clearance (CL/F) will be calculated using the formula dose/$AUC_{0\rightarrow inf}$. The apparent volume distribution (V/F) will be estimated by dividing CL/F by λ_z.

Outcome Measures

Differences in the geometric mean ratios (GMR) of the pharmacokinetic parameters will be compared between the two exposure conditions; i.e. *methylphenidate + CBD* vs *methylphenidate + placebo*.

Statistical Analysis: Results will be presented as median (range) for T_{max} and mean ± standard deviation (S.D.) for other data. The differences are considered statistically significant when the P values are less than 0.05.

7. PROTECTION OF HUMAN SUBJECTS

1. Risks to Human Subjects

a. Human Subjects Involvement and Characteristics:

Twelve (12) healthy men and women (50:50) between the ages of 18-50 years will complete this study. The number of prospective subjects will also allow for dropouts and failed screening evaluations. We anticipate a dropout/screening failure rate of approximately 5% based on past experience. All subjects will give written informed consent using a consent form approved by the University of Florida Institutional Review Board. Subjects will have to pass a screening evaluation based on history, physical examination and biochemical and urinalysis tests described above. Specific Inclusion and Exclusion criteria are outlined above under the *Study Population* section.

Abstinence from alcohol (in any form) for 24 hours prior to the screening visit as well as any of the scheduled study visits is required. No special populations, such as children less than 18 years of age, pregnant women, or prisoners will be included in this study. The racial and age mix of the study will be representative of the population of Alachua County, FL. There will be no exclusion based on race, sex, or ethnicity.

b. Sources of Materials: The following information will be collected from study participants for use in the research studies: demographic information (age, race/ethnicity, current medications, past medical history, and family history), body weight, height, history and physical exam, smoking status, and typical alcohol consumption. The laboratory data to be obtained from study participants include typical screening labs (i.e. complete blood count, liver function tests) and pregnancy tests for women with childbearing potential. Data will be kept in locked files that are only accessible to the investigators. During these studies, blood and urine samples will be collected during the health screening and the pharmacokinetic studies. The blood and urine will be obtained specifically for research purposes in order to meet our specific aims.

c. Potential Risks:

Venipuncture: The risks of drawing blood include temporary discomfort and inconvenience from the needle stick, and intravenous catheter placement. The procedures proposed have a potential for causing minor pain, bruising, bleeding, blood clots, and rarely, infection and swelling, and fainting could occur.

Dietary modification: During this study subjects will be asked to fast after 9 pm the evening prior to each of the two Full Study Day visits. Consumption of water during the fasting period is acceptable.

The most common side effects/adverse effects associated the two FDA-approved medications administered to the research subjects are as follows;

Cannabidiol (Epidiolex®): In the proposed study subjects will be take CBD 750 mg (or placebo) twice daily on their own for 3-Days in a run-up to the active Study Day at the CRC, and one 750 mg dose of CBD (or placebo) in the CRC. The 750 mg dose corresponds to a 10 mg/kg dose in a 70 kg adult. In a recently published report of the pharmacokinetics of Epidiolex® solution in healthy adult volunteers receiving 1500 mg twice daily (i.e. 2 x the dose proposed here) for 6 days, CBD was well-tolerated with the diarrhea, nausea, headache, somnolence and some instances of elevations in LFTs reported as the most common adverse events, all characterized as mild to moderate in severity.[13]

Although Epidiolex® prescribing literature recommends a dose titration in patients (2.5mg/kg bid x 7 days, 5mg/kg bid x 7 days, and then 10 mg/kg bid), there is precedent in the literature for *initiating* Epidiolex® at 10mg/kg bid or higher (as proposed here) in healthy adult volunteer pharmacokinetic and drug interaction assessments without issues of tolerability.[12.13]

Accordingly, we anticipate no untoward or serious adverse events associated with the short-term exposure to 750 mg bid of CBD in this study.

Liver Function Tests: Per Epidiolex® full prescribing literature, active treatment may cause dose-related elevations of liver transaminases (alanine aminotransferase [ALT] and/or aspartate aminotransferase [AST]). In controlled studies for indicated seizure disorders, the incidence of ALT elevations above 3 times the upper limit of normal (ULN) was 13% in Epidiolex®-treated patients compared with 1% in patients on placebo. Importantly, most of these studies involved patients also treated with other anticonvulsants (e.g. valproate) and involved exposures far longer than the 7 doses subjects will receive in the present study. Nevertheless, as an added safety measure, we will obtain baseline LFTs as part of the subject screening process and will repeat the LFT assessment after their last dose is received on the CRC unit.

Methylphenidate (Ritalin®): In the proposed study subjects will be given a single 10 mg dose of immediate-release *dl*-methylphenidate (Ritalin®) on two separate occasions at least 5 days apart. Methylphenidate has been in continuous clinical use for over 60 years and is the most commonly employed stimulant used to treat ADHD) in children and adults with approximately 18 million prescriptions dispensed annually in the US. Methylphenidate is well tolerated, has an excellent track record of safety, and serious adverse reactions are extremely rare. The most common side effects reported (with ongoing clinical treatment) are decreased appetite, stomachache, nervousness and insomnia. Insomnia associated with methylphenidate use is generally associated with taking late afternoon or evening doses. In the present study, a modest single 10 mg dose will be administered early in the morning. Clinically, immediate-release methylphenidate is routinely administered to children in doses of 60 mg/day or greater.

In the present study, the potential inhibition of methylphenidate metabolism (via CBD inhibition of CES1) is being assessed. In theory, if significant inhibition does occur, subjects would effectively be receiving a higher "dose" of methylphenidate than the 10 mg administered. However, even a doubling or tripling of the methylphenidate concentrations would still produce levels well within the range of clinically used dosing parameters (i.e. 60 mg/day and higher).
Moreover, he principal investigator (JSM) has performed numerous healthy volunteer studies in which methylphenidate was administered to healthy adult subjects in doses ranging from 10 mg immediate release to 40 mg sustained release with and without alcohol co-administration without any subject ever experiencing any serious adverse event. [14,15,16,17]

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent: Subjects will be recruited through advertisements that will be placed on bulletin boards (e.g. IRB-Approved Flyers). These sources of recruitment for healthy volunteers have been very successful in the past. Advertisements will be submitted to the University of Florida IRB for approval prior to use. Informed Consent will be obtained prior to any screening procedures by the PI or his designee after thorough discussion with the individual regarding the protocol, potential risks, and potential benefits of participation.

b. Protections Against Risk: The risk of adverse experiences in this study will be minimized by utilizing only qualified individuals to conduct the study, the staff in the UF Shands CRC. Appropriate attention to detail in the experimental setting will be emphasized. Moreover, this study will consist of single doses of two marketed botanical and vitamin formulations (i.e. one exposure to each formulation). Thus, the likelihood of formulation-associated adverse events should be minimized. Immediate medical treatment will be provided for any illness or injury resulting from this study. Trained nursing staff is present in the UF Shands CRC at all times and the physician co-investigator (Rajesh Mohandas, MD) will also be available to evaluate the subjects. In the event that a subject experiences an intolerable side effect, the subject will be withdrawn from the study immediately and followed to a satisfactory resolution of the effect(s). A subject may also be removed from the study if in the opinion of the physician investigator it is in the subject's best interest. Other risks of participation are minimal.

c. Potential Benefits of the Proposed Research to Human Subjects and Others
There is no direct benefit to the subjects as the aims of this research are to evaluate the potential drug interactions between CBD and methylphenidate with implications for other CES1 substrate medications (Table 1). No disease or condition is present or being treated. All procedures necessary for the completion of these studies will be performed at no cost to the

study subjects (or their insurance providers). There is some intrinsic value to the comprehensive physical assessment and health screen the subjects will receive, and copies of their laboratory results will be provided to subjects at their request.

All subjects will receive monetary compensation for study participation. Other than compensation for participation, there are no direct clinical benefits to the subjects for participating in this study.

Payment for Participation:
Subjects will receive a $500 at the completion of the entire research protocol. Parking at UF CRC is free. If it is determined during the initial screening procedure that a subject cannot participate in the study, each will be paid $25.00 for completing the screening procedure. If subjects are enrolled and elect not to complete the entire protocol for any reason, or are discontinued from the study by the investigators before protocol completion, each will receive partial compensation that will be pro-rated based upon the extent of their participation in the study to that point. For example, should a subject complete the first CRC study day for PK assessment and decide to discontinue the study, or be discontinued by the PI for any reason, they would be entitled to one half of the full study payment (i.e $250).

If subjects are paid for taking part in this study, their name and social security number will be reported to the appropriate university employees for the purpose of making and recording the payment. Research subjects will be responsible for paying income taxes on any payments provided by the study that total $600 or more or if the patient is a nonresident alien. Payment will be processed through the University of Florida Accounts Payable department and the University must report the amount received to the Internal Revenue Service (IRS).

8. Data and Safety Monitoring Plan

This study involves a small number of healthy volunteer subjects receiving single doses of a dietary supplement formulation who will be closely monitored by the Investigators and research personnel at the Shands CRC. All procedures will be performed in accordance with the Declaration of Helsinki on biomedical research involving human subjects as well as HIPPA. All biohazard materials such as blood samples will be handled and disposed in accordance with corresponding federal and state laws.

Study Limitations
Methylphenidate is an excellent representative probe substrate of CES1 and perturbations in its metabolism and disposition by CBD can reliably be attributed to this interaction mechanism under the described protocol. However, the results cannot necessarily be assumed to be

applicable to all CES1 substrates which might be co-administered with Epidiolex® solution. Nevertheless, it is accepted within the scientific and regulatory communities that clinical assessment utilizing probe drug methodology is a valid approach to provide general clinical insight into potential drug interaction liabilities beyond what *in vitro* studies can tell us.[18]

Finally, although not a limitation of the present study design per se, study results with Epidiolex® solution cannot be assumed to be applicable to other CBD formulations or CBD dosing route.

Conflict of Interest: The investigators DO NOT have a significant financial interest that would reasonably appear to be affected by the proposed research activities.

9. REFERENCES

1. https://clinicaltrials.gov/ct2/results?term=cannabidiol&Search=Search (Accessed 5/9/2019).
2. Chai G, Governale L, McMahon AW, Trinidad JP, Staffa J, Murphy D. Trends of outpatient prescription drug utilization in US children, 2002-2010. Pediatrics. 2012;130(1):23-31. Epub 2012/06/20. doi: 10.1542/peds.2011-2879. PubMed PMID: 22711728.
3. FDA Center for Drug Evaluation and Research. Epidiolex® (cannabidiol) Clinical Pharmacology and Biopharmaceutic(s) Review, Application #210365Orig1s000. Available at:https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210365Orig1s000ClinPharmR.pdf. Accessed August 23, 2018.
4. Qian Y, Wang X, Markowitz JS. In vitro inhibition of carboxylesterase 1 by major cannabinoids and selected metabolites. Drug Metab Dispos 2019; dmd.118.086074; DOI: https://doi.org/10.1124/dmd.118.086074
5. Zhu H-J, Patrick KS, Yuan H-J, Wang J-S, Donovan JL, DeVane, CL, Malcolm R, Johnson JA, Youngblood GL, Sweet DH, Langaee TY, Markowitz JS. Two CES1 gene mutations lead to dysfunctional carboxylesterase 1 activity in man: Clinical significance and molecular basis. Am J Hum Genetics 2008;82:1241-48. (PMID: 18485328)
6. Laizure SC, Herring V, Hu Z, et al. The role of human carboxylesterases in drug metabolism: have we overlooked their importance? Pharmacotherapy. Feb 2013;33(2):210-222.
7. Markowitz JS, Zhu H-J. Limitations of in vitro assessments of the drug interaction potential of botanical supplements. Planta Med 2012;78(13): 1421-1427
8. Zhu H-J, Patrick KS, Yuan H-J, Wang J-S, Donovan JL, DeVane, CL, Malcolm R, Johnson JA, Youngblood GL, Sweet DH, Langaee TY, Markowitz JS. Two CES1 gene mutations lead to dysfunctional carboxylesterase 1 activity in man: Clinical significance and molecular basis. Am J Hum Genetics 2008;82:1241-48. (PMID: 18485328)
9. Zhu H-J, Markowitz JS. Activation of the antiviral prodrug oseltamivir is impaired by two newly identified carboxylesterase 1 variants. Drug Metab Dispos 2009;37:264-7.

10. Gaston TE, Bebin EM, Cutter GR, Liu Y3, Szaflarski JP. Interactions between cannabidiol and commonly used antiepileptic drugs. Epilepsia. 2017 Sep;58(9):1586-1592.
11. Zhu HJ, Patrick KS, Markowitz JS. Enantiospecific determination of dl-methylphenidate and dl-ethylphenidate in plasma by liquid chromatography-tandem mass spectrometry: Application to human ethanol interactions. J Chromatogr B 2011;879:783-88. (PMID: 21931492)
12. Morrison G, Crockett J, Blakey G, Sommerville K. A Phase 1, open-label, pharmacokinetic trial to investigate possible drug-drug interactions between clobazam, stiripentol, or valproate and cannabidiol in healthy subjects. Clin Pharmacol Drug Dev. 2019 Feb 21. doi: 10.1002/cpdd.665.
13. Taylor L, Gidal B, Blakey G, Tayo B, Morrison G. A phase I, randomized, double-blind, placebo-controlled, single ascending dose, multiple dose, and food effect trial of the safety, tolerability and pharmacokinetics of highly purified cannabidiol in healthy subjects. CNS Drugs 2018;32:1053–1067
14. Markowitz JS, Logan BK, Diamond F, Patrick KS. Detection of the novel metabolite ethylphenidate following methylphenidate overdose with alcohol co-ingestion. J Clin Psychopharmacol 1999;19:362-6.
14. DeVane CL, Markowitz JS, Carson SW, Boulton DW, Gill HS, Nahas Z, Risch SC. Single dose methylphenidate pharmacokinetics in CYP 2D6 extensive and poor metabolizers. J Clin Psychopharmacol 2000;20:347-9
15. Markowitz JS, DeVane CL, Boulton DW, Nahas Z, Risch SC, Diamond F, Patrick KS. Ethylphenidate formation in human subjects after the administration of a single dose of methylphenidate and alcohol. Drug Metab Dispos 2000;28:620-24.
16. Markowitz JS, Straughn AB, Patrick KS, DeVane CL, Pestreich L, Lee J, Wang Y, Muniz R. Pharmacokinetics of methylphenidate after oral administration of two modified-release formulations in healthy adults. Clin Pharmacokinet 2003;42:393-401.
17. Zhu HJ, Patrick KS, Straughn AB, Reeves OT, Bernstein H, Shi, H, Johnson HJ, Knight, JM, Smith AT, Malcolm RJ, Markowitz JS. Ethanol interactions with dexmethylphenidate and dl-methylphenidate spheroidal oral drug absorption systems in healthy volunteers. J Clin Psychopharmacol 2017;37:419-428
18. Gurley BJ, Markowitz JS, Williams DK, Barone GW. Practical considerations when designing and conducting clinical pharmacokinetic botanical-drug interaction studies. Int J Pharmacokinetics 2017;2(1):57-69

APPENDIX

Laboratory Studies for Health Screening

Comprehensive Metabolic Panel

Glucose

Creatinine

BUN

AST

ALT

Alkaline phosphatase

Total bilirubin

Sodium

Potassium

Chloride

Calcium

Total protein

Albumin

CBC with Differential

Urine THC Screen