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RESEARCH ARTICLE

A study on CYP2C9 polymorphism in Puerto Rican Alzheimer's Patients and its role in the Pharmacokinetics of Δ-9-tetrahydrocannabinol

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects over 55 million people worldwide. Individuals with AD are often prescribed multiple medications to manage comorbidities, many of which are metabolized by enzymes from the cytochrome P450 (CYP). CYP2C9 is expressed by the CYP2C9 gene. The highly polymorphic gene is grouped into phenotypes such as poor, intermediate, normal, and ultra-rapid metabolizers that influence the pharmacokinetics (PK) of drugs and their metabolites. Among humans, CYP2C9 is one of the most essential enzymes for metabolizing drugs, including delta-9-tetrahydrocannabinol (THC). The enzyme converts THC into its active metabolite 11-hydroxy-delta-9-THC (OH-THC). IGC-AD1 is a formulation with two active pharmaceutical ingredients, delta-9-THC and melatonin. The Phase 1, multiple ascending dose (MAD) trial, was conducted on a Puerto Rican population. The participant population in the trial (N=13) was 69.2% female and 30.8% male, with an average age of 80.18 (SD+/- 6.22). We report on the effect of CYP2C9 polymorphisms on the pharmacokinetics (PK) of THC and its active metabolite in AD patients from a Phase 1 trial. Using a Mass ARRAY Analyzer 4 Instrument (Invitae Inc.), we determined the following CYP2C9 alleles: *2, *3, *4, *5, *6, *8, *11, *13, *15, and found that 60% of participants (N=6) were carriers of at least one polymorphism including 1*/2* and 1*/3*. The participants with intermediate metabolizers (*1/*3 and *1/*2) showed an increased half-life of THC and OH-THC with major differences between the two intermediate metabolizer groups *1/*3 and *1/*2. In the trial more females were noted to be intermediate metabolizers than males. As polymorphisms of CYP2C9 affect the PK of THC and its metabolite, larger studies are needed to establish PK baselines for polymorphisms of CYP2C9. In the meantime, it is recommended that researchers exercise caution while dosing AD patients with THC.

Keywords: Alzheimer's disease, Genotyping, CYP2C9, THC, Pharmacokinetics, Intermediate metabolizer, Latino population

Introduction

Alzheimer's disease (AD) is a chronic neurogenerative disease, with more than 55 million people living with it worldwide[1]. The predicted cost of treating AD in healthcare in 2024 is around $350 billion, and by 2050, prices are expected to surpass $1 trillion[2]. AD is characterized by the presence of pathological hallmarks such as Aβ plaques, neurofibrillary tangles, and neuronal loss. These pathological changes are associated with cognitive impairment, and behavioral and neuropsychiatric symptoms. Cognitive impairment due to AD progression includes memory loss, language difficulties, and altered executive function. AD patients also exhibit neuropsychiatric symptoms, collectively known as behavioral and psychological symptoms of dementia (BPSD). BPSD includes agitation, anxiety, elation, irritability, depression, apathy, disinhibition, aberrant motor behavior, delusions, hallucinations, sleep and appetite changes[3]. People with AD often live with more comorbidities such as diabetes, heart disease, and respiratory conditions compared to their age-matched counterparts without dementia[4]. An earlier study indicated that patients who developed AD had significantly higher increases in long-term comorbidities compared to their age-matched control in the following conditions: hypertension (AD: 69.7% vs. Control: 65.32%), diabetes (AD: 32.7% vs. Control: 25.1%), lung disease (AD: 15.4% vs Control: 12.2%), and heart problems (AD: 38.7% vs. Control: 28.9%)[5]. Due to the complex nature of disease symptoms, patients are often prescribed multiple medications to manage both AD-related symptoms and comorbidities. These medications may include cholinesterase inhibitors, antidepressants, antipsychotics, antidiabetics, and antihypertensive drugs, among others[6].

Inter-individual variability in medication response is frequently seen in clinical practice. This variability can result in reduced therapeutic effectiveness or increased risk of adverse drug reactions, which can substantially impact patient's health and be a healthcare system burden. This variability in drug response is partly due to variations in drug metabolism. Enzymes such as the cytochrome (CYP) P450 are responsible for processing almost all drugs that enter the body. CYP enzymes are membrane-bound hemoproteins that play crucial roles in drug detoxification, cellular metabolism, and homeostasis. The majority of clinical drug metabolism is carried out mostly by isoforms of the CYP1, 2, and 3 families[7]. CYPs converts lipophilic drugs into hydrophilic metabolites to facilitate their elimination, further influencing drug action, safety, bioavailability, and drug resistance through metabolism in the liver and in local sites of action. This process has a significant impact on treatment outcomes[8]. Concomitant medications and circulating metabolites have the potential to inhibit or induce CYPs, which may have an impact on treatment outcomes through drug-drug interactions, drug-gene interactions, and drug-drug–gene interactions [9]. The CYP gene polymorphisms contribute to several allelic variations, the frequency of which ranges in various populations[10]. Based on genetic variances in CYP genes, four types of phenotypic alterations in CYPs have now been identified: poor metabolizers, extensive metabolizers, intermediate metabolizers, and ultra-rapid metabolizers. These phenotype changes could impact drug response and therapeutic outcomes[11].

Cytochromes 2C8, 2C9, 2C18, and 2C19 are the four enzymes that make up the CYP2C subfamily. Among these, CYP2C9 has the highest level of expression and makes the most contribution to drug metabolism, while it catalyzes many exogenous and endogenous substances that may be substrates for other phase I or Phase II enzymes[15]. Mass spectrometry quantification indicates that CYP2C9 contributes around 20% of the total hepatic P450[12]. In terms of the quantity of therapeutic compounds oxidized, CYP2C9 is the third most significant cytochrome P450, accounting for 25% of all medications that undergo P450-catalyzed biotransformation[13,14,15]. This is behind CYP3A4 and CYP2D6[13,14]. The CYP2C9 gene is highly polymorphic, with 61 alleles reported, all of which have reduced or no enzymatic activity. CYP2C9 variant alleles are grouped into tier-1 and tier-2 by the Association for Molecular Pathology Pharmacogenomics based on their functional effects over CYP2C9 activity and drug response, availability of reference materials, as well as their considerable allele frequencies in major ethnic groups[16]. CYP2C9 tier-1 is comprised of CYP2C9 *2, *3, *5, *6, *8, *and* *11 and tier-2 consist of CYP2C9 *12, *13 and *15.

Delta-9-tetrahydrocannabinol (THC) is absorbed well in the gastrointestinal system[17]. Orally given THC has a limited bioavailability of about 2–20% due to substantial first-pass metabolism. Among humans, 11-hydroxy-Δ9 THC (OH-THC) is the primary metabolic pathway, mainly via CYP2C9, followed by CYP3A4 and CYP2C19[18-20], showing notable psychoactive properties as a metabolite. After further oxidation, OH-THC becomes the pharmacologically inactive compound 11-nor-9-carboxy-Δ9 THC (COOH-THC). Plasma melatonin is metabolized by hepatic cytochrome P450 enzymes, mainly by the 6-hydroxylating sub-form CYP1A2[18-20, 21].

High interindividual variability influences the plasma concentration of cannabinoids and their metabolites, and this variability is reflected in the control of cannabinoid compounds in plasma levels by the CYP2C9 enzyme[18]. In this study, we assessed an exploratory investigation on the CYP2C9 polymorphism and its effect on THC pharmacokinetics (PK) in an AD Puerto Rican (PR) sample (n = 10) as a safety precaution with our FDA-regulated product in a Phase 1 trial. IGC-AD1 is an oral formulation composed of THC and melatonin. Based on earlier studies, the population showed a polymorphism of CYP2C9 in Caribbean Hispanics from Puerto Rico[22]. We present the PK of THC and OH-THC for each of the genotypes of CYP2C9 found in the trial.

Methods

STUDY DESIGN

A Multiple Ascending Dose (MAD) Phase 1 double blind, randomized placebo-controlled trial (ClinicalTrials.gov ID: NCT04749563) was conducted to assess the safety and tolerability of IGC-AD1 in patients with AD. As an exploratory investigation, the pharmacokinetics and genotyping of CYP2C9 alleles were studied. All the participants (Active; n=11 and Placebo; n=2) gave their written informed consent prior to admission to the study. For the analysis, 10 active participants were considered. The demographic data of the participants are given in Table 1. The study was conducted in accordance with the principles of the Declaration of Helsinki. The protocol was approved by Advarra Ethics Committee, Columbia, MD. For the PK study, all active participants (n=10) received a single oral dose (1mL) of IGC-AD1 containing THC (2.5mg/mL) and melatonin (1.5mg/mL).

Table 1. Demographic Data of Puerto Rican AD Patients

IGC-AD1 Actives	Males, n (%)	Female, n (%)	Age (years ± SD)	Ethnicity	Race White, n (%)	Race Black, n (%)
N=10	3 (30%)	7 (70%)	80.4±6.22	Hispanic or Latino: Puerto Rican	7 (70%)	3 (30%)

GENOTYPING

Procedures for genotyping are currently used to predict CYP activity. Cytochrome genotyping relies on DNA analysis and the estimation of enzyme activity from the detected alleles. This method allows the identification of the single nucleotide polymorphisms allele. For the Cytochrome 2C9 polymorphism genotyping, a 4 ml blood sample was taken from each participant in a K_2EDTA tube and stored at 2-8° C. Samples were shipped to the laboratory on dry ice and kept at -70° C until analysis. Blood samples were evaluated at Invitae Corporation, CA., USA. The genotyping laboratory was a CLIA-certified laboratory that met the standards as per the FDA guidelines. Briefly, the assay was conducted on the Mass ARRAY Analyzer 4 Instrument (MAA4, Agena Biosciences, San Diego, CA) and involved a single base extension using mass-modified dideoxynucleoside terminators of an oligonucleotide primer that anneals just upstream of the polymorphic site of interest after a first locus-specific polymerase chain reaction. The MALDI-TOF mass spectrometry was employed to determine the unique mass of the extended primer that identifies the single nucleotide polymorphisms allele. The presence of alleles is represented by *1/*1 as a normal metabolizer.

BLOOD SAMPLE COLLECTION FOR PK STUDIES

The pharmacokinetics of the THC measurement method involves administering orally 1 ml of IGC-AD1 comprising 2.5 mg of THC. The distribution of THC is calculated in the plasma by measuring the THC and its metabolite using liquid chromatography separation and detected by sophisticated mass spectroscopy detection method. Blood was collected into a 4 ml K_2EDTA blood tube. The plasma was immediately separated by centrifugation at 3000 rpm for 10 minutes and kept at -20°C. Samples were then shipped to the laboratory on dry ice and kept at -70°C until analysis. Blood draws were collected at the following time intervals: T minus 15 minutes (T-15), T+ 1, T+2, T+2.5, T+3, T+3.5, T+4, T+5, T+12, T+14, and T+16 (hours), where T=0 represents dosing.

PK ANALYSIS

Using a reverse phase liquid chromatography separation and detection by in-source fragmentation and tandem mass spectrometer (LC-MS/MS), the analysis was conducted at Northeast Biolab, (Hamden, CT 06518). The lower limit of quantification (LLQ) was established at (±) 0.20 ng/ml for THC and (±) 0.50 ng/ml for -11-0H-D9-THC. The FDA (2018) Bioanalytical Method Validation and FDA Good Laboratory Practice

(GLP, Title 21 CFR Part 58) were followed in the method's validation for THC and its metabolite 11-0H-D9-THC. The data from every participant complied, and the Phoenix Win Nonlinear software version was used to conduct a non-compartmental analysis to obtain the Half-life ($t_{1/2}$; h), the maximum concentration (C_{max}; ng/mL), the time to peak measure (T_{max}; h), the area under the curve (AUC; h*ng/mL) last, and AUC infinity values (AUC_{inf}; h*ng/mL). The values are expressed in ng/mL.

Data analysis: Values are expressed in mean and standard deviation (SD). The level of difference in genotyping alleles are expressed in percentages. PK variables were estimated using Phoenix Win Nonlinear software and summarized using descriptive statistics.

Results

GENOTYPING

We determined the presence of CYP2C9*1, *2, *3, *4, *5, *6, *8, *11, *13, *15 in the participants from the IGC-AD1 group. The study revealed that 60% of participants (n = 6) had a CYP2C9 gene polymorphism of intermediate metabolizer (Table 2). Out of 60% of participants, 40% of participants (n = 4) had CYP2C9 *1/*2 variant with intermediate metabolizer alleles, and 20% of participants (n = 2) showed CYP2C9 *1/*3 variant with intermediate metabolizer alleles. The remaining 40% of participants in the sample (n = 4) had wild-type normal CYP2C9 *1/*1 alleles. The presence of intermediate metabolizer alleles can contribute to the reduced activity of the CYP2C9 enzyme (Table 2)

Table 2. CYP2C9 Polymorphism in Puerto Rican AD Patients

Participants	CYP2C9 Genotype	CYP2C9 Phenotype	Sex	Metabolizer
1	*1/*2	Intermediate Metabolizer	Female	60% **Intermediate Metabolizer**
2	*1/*2	Intermediate Metabolizer	Female	
3	*1/*3	Intermediate Metabolizer	Female	
4	*1/*2	Intermediate Metabolizer	Female	
5	*1/*3	Intermediate Metabolizer	Male	
10	*1/*2	Intermediate Metabolizer	Male	
6	*1/*1	Normal Metabolizer	Male	40% **Normal Metabolizer**
7	*1/*1	Normal Metabolizer	Female	
8	*1/*1	Normal Metabolizer	Female	
9	*1/*1	Normal Metabolizer	Female	

PK OF THC

The pharmacokinetics of THC at low concentrations and its active metabolite 11-0H-D9-THC in AD patients were measured at different time intervals. The study showed a high inter-variability among the participants in PK parameters. After a single dose of IGC-AD1 (2.5 mg THC), the normal metabolizers (n=4) showed a C_{max} for THC and its metabolite 11-0H-D9-THC of 2.60 and 3.53 ng/mL, respectively, the T_{max} was 2.38 and 1.75 hours for THC and its metabolite respectively. The $T_{1/2}$ for THC and its metabolite were 1.74 and 2.98 hours, respectively. The AUC to infinity for THC and its metabolite were 9.04 ng*h/mL and 16.40 ng*h/mL respectively. The AUC ratio of THC to metabolite was 0.55 ng*h/mL.

POLYMORPHISMS EFFECTS ON PK

The PK parameters for the intermediate metabolizer patients showed differences compared to the normal metabolizer group. The intermediate metabolizers *1/*2 and *1/*3 showed lower THC C_{max} ng/mL values of 1.46 and 1.93, respectively, compared to normal metabolizers (THC C_{max} = 2.60 ng/mL). However, THC metabolite11-0H-D9-THC value was increased in CYP2C9 *1/*3, with a C_{max} value of 7.73 ng*h/mL suggesting decreased activity in *1/*3 alleles that could possibly contribute to decreased clearance of THC metabolite. There was a slight change in T_{max} in both THC and its metabolite in the intermediate metabolizer groups (Table 3a & b).

Table 3a. CYP2C9 Polymorphism Effect on PK of THC

THC	Mean over All	Mean over *1/*1	Mean over *1/*2	Mean over *1/*3
No. Patients	N=10	Normal (n=4)	Intermediate (n=4)	Intermediate (n=2)
$T_{1/2}$ (h)	3.60 ± 4.09 (n=9)	1.74 ± 1.50	3.75 ± 3.30 (n=3)	7.10 ± 8.17
T_{max} (h)	2.15 ± 1.03	2.38 ± 0.95	2.13 ±1.31	1.75 ± 1.06
C_{max} (ng/mL)	2.00 ± 1.19	2.60 ± 1.71	1.46 ± 0.71	1.93 ± 0.0
AUC_{last} (h*ng/mL)	5.58 ± 2.78	6.72 ± 3.09	4.20 ± 2.93	6.06 ± 1.45
AUCinf (h*ng/mL)	8.92 ± 3.93 (n = 9)	9.04 ± 3.70	7.24 ± 3.23 (n = 3)	11.22 ± 6.51

Data are expressed in Mean and ± SD.

Table 3b. CYP2C9 Polymorphism Effect on PK of 11-0H-D9-THC

OH-THC	Mean over	Mean over *1/*1	Mean over *1/*2	Mean over *1/*3
No. Patients	**N=10**	**Normal (n=4)**	**Intermediate (n=4)**	**Intermediate (n=2)**
$T_{1/2}$ (h)	3.30 ±1.61	2.98 ± 1.95	3.35 ± 1.68	3.84 ± 1.53
T_{max} (h)	1.90 ± 0.99	1.75 ± 0.96	2.00 ± 1.41	2.00 ± 0.0
C_{max} (ng/mL)	4.30 ± 3.57	3.53 ± 1.77	3.35 ± 1.20	7.73 ± 8.45
AUC_{last} (h*ng/mL)	17.03 ±19.00	12.30 ± 7.8	12.51 ± 11.6	35.53 ± 42.95
AUCinf (h*ng/mL)	21.60 ± 19.08	16.40 ± 7.15	17.73 ± 12.35	39.75 ± 42.86

Data are expressed in Mean and ± SD.

Interestingly, the half-life of THC and its metabolite in both intermediate metabolizer groups showed an increase in half-life compared to the normal metabolizers (Table 3a & b). Due to the delayed clearance with increased half-life caused by the presence of polymorphic CYP2C9 *1/*2 and *1/*3, AUC levels of the THC metabolite were higher in the CYP2C9 *1/*3 carrier group compared to the normal metabolizer and intermediate *1/*2 metabolizer groups (Table 3a & b). Overall, the presence of polymorphic alleles was related to a reduced clearance of THC metabolite in plasma. The PK of THC and its metabolite in women (n=7) showed larger, two-fold, AUC to infinity compared to males (n=3, Table 4a & b).

Table 4a. Sex Influence on Metabolism of THC

Sex	$T_{½}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{last} (h*ng/mL)	AUC_{inf} (h*ng/mL)
Male (n =3)	1.50 ± 0.61	2 ± 0.87	1.63 ± 0.29	4.15 ± 1.78	5.32 ± 1.60
Female (n=7)	4.65 ± 4.76 (n = 6)	2.21 ±1.15	2.17 ± 1.42	6.19 ± 3.02	10.73 ± 3.46 (n = 6)

Data are expressed in Mean and ± SD

Table 4b. Sex Influence on Formation of OH-THC

Sex	$T_{½}$ (h)	T_{max} (h)	C_{max} (ng/mL)	AUC_{last} (h*ng/mL)	AUC_{inf} (h*ng/mL)
Male (n =3)	2.05 ± 0.62	1.67 ± 0.58	2.47 ± 0.75	6.57 ± 1.50	9.34 ± 0.35
Female (n=7)	3.83 ± 1.62	2 ± 1.15	5.08 ± 4.07	21.52 ± 21.51	26.86 ± 20.94

Data are expressed in Mean and ± SD.

Discussion

The present study demonstrates a high prevalence of CYP2C9 polymorphism (CYP2C9 *1/*2 and*1/*3) among Puerto Ricans, which affects the PK of THC and its metabolite. High frequencies of the CYP2C9*2 and CYP2C9*3 alleles have been seen in white Europeans[14], and low rates in African Americans (1–2%) and most Asians, indicating that these variations may be of little or no relevance in the latter populations. In addition, the study found that sex can affect the metabolism of THC and the formation of OH-THC.

The most prevalent isoform in the liver, CYP2C9 is responsible for the metabolism of many medications, including phenytoin and warfarin, as well as a variety of non-steroidal anti-inflammatory medications and antidiabetic medications like tolbutamide and glipizide[23,24]. Those studies imply that adjustments in oral anticoagulant and other medication doses are needed across individuals with genetic variation in the CYP2C9. Similarly, 60% of the current study participants on active medication showed variations in either CYP2C9 *1/*2 and*1/*3 genes. Earlier studies had shown the presence of polymorphism in CYP2C9*5, CYP2C9*8, CYP2C9*9, CYP2C9*11, CYP2C9*12, CYP2C9*21, and CYP2C9*61 in Caribbean Hispanics from Puerto Rico[22]. We did not see variation in those alleles in our participant samples. However, the genetic variation observed in the results suggested that the presence of CYP2C9 polymorphism could have an effect in the metabolism of THC and other clinically relevant drugs metabolized by this enzyme.

Earlier *in vitro* studies had shown that the presence of CYP2C9 *1/*2 polymorphism diminishes 50–70% of enzyme activity, while CYP2C9 *1/*3 polymorphism nearly drops enzyme function (reduction of 75–99%)[25,26]. According to earlier studies, CYP2C9 polymorphisms change the synthesis of 11-OH-THC, an active metabolite, and 11-COOH-THC, an inactive component. Reduced 11-COOH-THC and high THC concentrations can make people more susceptible to adverse psychoactive effects [27]. The current study showed a two-fold increase in AUC levels of active THC metabolite 11-0H-D9-THC in CYP2C9 *3* polymorphic carriers compared to normal metabolizers. Earlier studies have shown that CYP2C9*3 carriers tend to become drowsier after taking 15 mg of THC [28]. In our study, we did not observe sedation among AD patients. This may be due to the lower doses of THC (2.5 mg/mL) present in the IGC-AD1. This is due to the presence of CYP2C9 *1/*2 and CYP2C9 *1/*3, which increased the half-life of THC as well as its active metabolite compared to normal metabolizers. The data from this study also suggests that the sex of participants influences THC metabolism in AD patients. This finding is consistent with earlier studies on the influence of THC metabolism after acute and repeated dosing of THC in animals[29,30] as well as in humans[31]. This alteration is attributed to females metabolizing THC at a faster rate than males[32].

Most people with poor metabolizer phenotypes are more likely to experience psychosis and memory loss, mainly when using THC at greater dosages or for more extended periods [16,33]. In the current study, even though 60% of the participants showed CYP2C9 polymorphism, we did not observe severe adverse effects, probably due to the low dose of THC administration. In addition, no adverse events led to any participants withdrawing from the trial. Our findings suggest that a high prevalence of CYP2C9 polymorphism affects the PK of THC.

The limitation of this current study is a smaller sample size of participants from one region. Future studies should consider demographics from all races and a larger sample size will provide a greater understanding of CYP2C9 polymorphism effects on THC and other clinically relevant drug metabolism and its adverse effects.

Conclusions

In conclusion, the Puerto Ricans who participated showed a high prevalence of polymorphisms in CYP2C9 that affect the PK of THC and its active metabolite OH-THC. It was also observed that sex plays a role in the metabolism of THC and its metabolite. Determining CYP2C9 polymorphisms before THC dosing may provide additional information on the drug efficacy and toxicity in the general population. Physicians and researchers are advised to exercise caution and consider CYP2C9 polymorphisms when dosing with THC in AD patients.

Conflicts of Interest Statement

The authors are IGC Pharma employees. Dr. William Julio was the Principal Investigator for the study.

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