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ORIGINAL RESEARCH

Comparative Pharmacokinetics of Δ9-Tetrahydrocannabinol in Adolescent and Adult Female Mice

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Abstract

Introduction: Animal studies suggest that adolescent exposure to Δ^9-tetrahydrocannabinol (Δ^9-THC), the intoxicating constituent of cannabis, causes lasting functional alterations in brain and other organs. Those studies often neglect the impact that age- and sex-dependent differences in the distribution and metabolism of the drug might exert on its pharmacological effects. Here, we provide a comparative analysis of Δ^9-THC pharmacokinetics in adolescent and adult female mice, which identify significant dissimilarities in distribution and metabolism of Δ^9-THC between females of these age groups.

Materials and Methods: We administered Δ^9-THC (5 mg/kg, intraperitoneal) to adolescent (37-day old) and young adult (70-day old) female mice and quantified Δ^9-THC and its first-pass metabolites—11-hydroxy-Δ^9-THC (11-OH-THC) and 11-nor-9-carboxy-Δ^9-THC (11-COOH-THC)—in plasma and brain tissue using liquid chromatography/tandem mass spectrometry.

Results: Maximal plasma concentrations of Δ^9-THC were 8 times higher in adolescent than adult female mice. Conversely, brain concentrations and brain-to-plasma ratios were 25–50% higher in adults than adolescents. Concentrations of Δ^9-THC metabolites were higher in plasma but lower in brain of adolescent compared to adult female mice.

Conclusions: The results identify multiple age-dependent differences in the pharmacokinetic properties of Δ^9-THC in female mice, which might influence the pharmacological response to the drug.

Keywords: Δ^9-Tetrahydrocannabinol; cannabis; pharmacokinetics; liquid chromatography/tandem mass spectrometry (LC-MS/MS)

Introduction

Frequent cannabis use during adolescence has been associated with neuropsychological and metabolic changes in adulthood.[1–3] Frequency, duration, and age of onset are important factors in determining the long-term effects of adolescent cannabis use.[4] However, results have been inconsistent across studies and further research is needed to elucidate the effects of cannabis in adolescents compared with adults. In an effort to understand the influence of age, sex, and mammalian species on Δ^9-tetrahydrocannabinol (Δ^9-THC) pharmacokinetics, the psychotropic component of cannabis, we previously investigated Δ^9-THC pharmacokinetics in adolescent and adult male mice[5] and in adolescent and adult rats of both sexes.[6] In male mice, we found two differences between younger and older animals: (1) adolescent males preferentially metabolize Δ^9-THC into its inactive metabolite 11-nor-9-carboxy-Δ^9-THC (11-COOH-THC); and (2) the brain of adolescent male mice is partly protected from Δ^9-THC[5]. By contrast, in rats, we found strong sex-dependent but not age-dependent differences: regardless of age, female rats metabolize Δ^9-THC to its bioactive metabolite, 11-hydroxy-Δ^9-THC (11-OH-THC), more effectively than males do.[6]

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To determine if the age-dependent differences seen in male mice occur also in females, in the present study we carried out a pharmacokinetic analysis of Δ^9-THC in pubertal adolescent (37 day-old) and young-adult (70 day-old) female mice. We administered the drug by intraperitoneal (IP) injection and quantified Δ^9-THC and its main cytochrome P450 metabolites, 11-OH-THC and 11-COOH-THC, in plasma and brain using isotope-dilution liquid chromatography/tandem mass spectrometry (LC-MS/MS).[7] The results reveal the existence of significant dissimilarities in the pharmacokinetic profile of Δ^9-THC between adolescent and adult female mice, which demonstrate notable species-related differences between mice and rats.

Materials and Methods

Chemicals and solvents

$[^2H_3]$-THC, $[^2H_3]$–11-OH-THC, and $[^2H_3]$–11-COOH-THC were purchased from Cerilliant (Round Rock, TX). Δ^9-THC was from Cayman Chemicals (Ann Arbor, MI). All analytical solvents were of the highest grade, and were obtained from Honeywell (Muskegon, MI) or Sigma-Aldrich (Saint Louis, MO). Formic acid was from Thermo Fisher (Houston, TX).

Animals

Adolescent (postnatal day, PND, at arrival: 22; 15–20 *g*) and adult (PND 60, 20–25 *g*) female C57BL/6 mice were purchased from Charles River (Wilmington, MA). They were housed in groups of 4 and were allowed to acclimate for at least 3 days before experiments. Housing rooms were maintained on a 12-h light/12-h dark cycle (lights on at 6:30 AM) under controlled conditions of temperature (20 ± 2°C) and relative humidity (55%–60%). Food and water were available *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee at the University of California, Irvine, and carried out in strict accordance with the National Institutes of Health guidelines for the care and use of experimental animals.

Pharmacokinetic experiments

We dissolved Δ^9-THC in Tween80/saline (5:95, v/v) and administered it at 5 mg/kg by IP injection in a volume of 10 mL/kg. The mice were anesthetized with isoflurane at various time points after injection ($n = 4$ per timepoint), blood was collected by cardiac puncture into ethylenediamine-tetraacetic acid (EDTA)-rinsed syringes and transferred into 1 mL polypropylene tubes containing spray-coated potassium-EDTA (K2-EDTA). Plasma was prepared by centrifugation at 1450×*g* at 4°C for 15 min and transferred into polypropylene tubes. The mice were decapitated, and their brains quickly removed. All tissue samples were immediately frozen on dry ice and stored at −80°C until analyses.

Sample preparation

Plasma (0.1 mL) was transferred into 8 mL glass vials (Thermo Fisher), and proteins were precipitated by addition of 0.5 mL of ice-cold acetonitrile containing 1% formic acid and the after internal standards (ISTD): $[^2H3]$-THC, $[^2H3]$–11-OH-THC, and $[^2H3]$–11-COOH-THC, 50 pmol each. Frozen whole brains were pulverized on dry ice. Tissue samples (20–25 mg) were homogenized using a Precellys CK-14 homogenizing kit (Bertin Corp., Rockville, MD) in a Precellys Evolution apparatus (Bertin) at 4°C in 0.5 mL of ice-cold acetonitrile containing 1% formic acid and 50 pmol ISTD. Plasma and brain samples were stirred vigorously for 30 s and centrifuged at 2800×*g* at 4°C for 15 min. After centrifugation, the supernatants were loaded onto captiva-enhanced matrix removal (EMR)-Lipid cartridges (Agilent Technologies, Santa Clara, CA) and eluted under positive pressure (3–5 mmHg, 1 drop/5 sec). For brain fractionation, EMR cartridges were pre-washed with water/acetonitrile (1:4, v/v). No pretreatment was necessary for plasma. Tissue pellets were rinsed with water/acetonitrile (1:4, v/v; 0.2 mL), stirred for 30 s, and centrifuged at 2800×*g* at 4°C for 15 min. The supernatants were collected, transferred onto EMR cartridges, eluted, and pooled with the first eluate. The cartridges were washed again with water/acetonitrile (1:4, v/v; 0.2 mL), and pressure was increased gradually to 10 mmHg (1 drop/sec) to ensure maximal analyte recovery. Eluates were dried under N2 and reconstituted in 0.1 mL of methanol containing 0.1% formic acid. Samples were transferred to deactivated glass inserts (0.2 mL) placed inside amber glass vials (2 mL; Agilent).

Liquid chromatography/mass spectrometry analyses

LC separations were carried out using a 1200 series LC system coupled to a 6410B triple quadrupole mass spectrometric detector (MSD; Agilent). Analytes were separated on an Eclipse XDB C18 column (1.8 μm, 3.0 × 50.0 mm; Agilent). The mobile phase consisted of water containing 0.1% formic acid as solvent A and methanol containing 0.1% formic acid as solvent B.

The flow rate was 1.0 mL/min. Gradient conditions were as follows: starting 75% B to 89% B in 3.0 min, changed to 95% B at 3.01 min, and maintained till 4.5 min. Equilibration time was 2.5 min. Column temperature was 40°C and autosampler temperature 9°C. Injection volume was 5 μL. The MS was operated in the positive electrospray ionization mode, and analytes were quantified by multiple reaction monitoring of the after transitions: Δ9-THC 315.2 > 193.0 *m/z*, [^{2}H3]-THC 318.2 > 196.1 *m/z*, 11-OH-THC 331.2 > 313.1 *m/z*, [^{2}H3]–11-OH-THC 334.2 > 316.1 *m/z*, 11-COOH-THC 345.2 > 299.2 *m/z*, [^{2}H3]–11-COOH-THC 348.2 > 302.2 *m/z*. The identity of Δ9-THC, 11-OH-THC and 11-COOH-THC was verified by monitoring the transitions 315.2 > 123.0 *m/z*, 331.2 > 105.0 *m/z* and 345.2 > 299.2 *m/z*, respectively. Capillary voltage was 3500 V. Source temperature was 300°C and gas flow was 12.0 L/min. Nebulizer pressure was 40 psi. The MassHunter software (Agilent) was used for instrument control, data acquisition, and data analysis.

Pharmacokinetic data analyses

Pharmacokinetic data were analyzed using a non-compartmental model[10] as described previously.[5–9]

Statistical analyses

Data were analyzed by Student's unpaired *t*-test or two-way analysis of variance with Bonferroni *post hoc* test using GraphPad Prism 8. Outliers were determined using the Grubbs' outlier test and removed if they were above or below the threshold (alpha = 0.05). Differences between groups were considered statistically significant at values of $p < 0.05$.[9]

Results

Plasma pharmacokinetics

Figure 1A–B shows the plasma profile of Δ^9-THC and its bioactive cytochrome P450 metabolite, 11-OH-THC, in young adult (PND 70) and adolescent (PND 37) female mice after IP administration of 5 mg/kg Δ^9-THC (n = 3 or 4 animals per timepoint). Table 1 reports peak plasma concentration (C_{max}), area under the curve (AUC), time at which C_{max} was attained (T_{max}) and apparent half-life time ($t_{1/2}$) of elimination for each analyte including 11-COOH-THC. In adults, C_{max} for Δ^9-THC was 1711 ± 790 pmol/mL (mean ± SEM) at a T_{max} of 15 min. C_{max} for 11-OH-THC and 11-COOH-THC were 89 ± 9 pmol/mL and 192 ± 78 pmol/mL, respectively, and were reached 30 and 60 min after injection. Apparent plasma $t_{1/2}$ was 48.1 ± 5 min. Apparent volume of distribution (V_D) of Δ^9-THC in adult female mice, calculated using a non-compartmental model,[10] was 186 ± 41 mL (λ_z = 0.015 ± 0.003), and clearance (CL) was 2.7 ± 0.1 mL/min.

Similar plasma profiles were observed in adolescent (PND 37) female mice, with some notable exceptions (Fig. 1A–B and Table 1). The C_{max} for Δ^9-THC was 8 times higher in adolescent than adult animals. Moreover, C_{max} values for 11-OH-THC and 11-COOH-THC were 3.5- and 1.5-times higher in adolescents than adults, respectively (316 ± 40 *vs.* 89 ± 9 pmol/mL, $p < 0.05$, 11-OH-THC; 299 ± 18 *vs.* 192 ± 78 pmol/mL, ns, 11-COOH-THC). Elimination was faster in adolescents with a $t_{1/2}$ of 33.9 ± 0.2 min. Δ^9-THC V_D in adolescents was 40 ± 2 mL (λ_z = 0.019 ± 0.001), and CL was 0.8 ± 0.03 mL/min, and both were lower ($p <$ 0.001) than the corresponding values in adults, providing a possible explanation for the higher peak concentrations of Δ^9-THC found in plasma of younger mice.

Brain pharmacokinetics

The profiles of Δ^9-THC and 11-OH-THC in brain of adult and adolescent female mice are illustrated in Figure 1C–D. Key parameters are reported in Table 2. In adults, Δ^9-THC C_{max} was 550 ± 140 pmol/g, the T_{max} was 60 min, and apparent $t_{1/2}$ of elimination was 48.7 ± 3 min. Of note, brain-to-plasma ratio of Δ^9-THC was relatively low (1.08 ± 0.2) but consistent with values previously reported in the literature.[11,12] 11-OH-THC and 11-COOH-THC C_{max} was 567 ± 153 at 60 min and 210 ± 33 pmol/g at 120 min, respectively (Table 2).

Comparing data in Figure 1C–D shows that brain Δ^9-THC C_{max} was 1.6 times lower in adolescent than adult female mice. AUC was also 1.5 times lower in the younger group (Table 2). Further, C_{max} and AUC values for Δ^9-THC metabolites were either significantly lower or showed trends to be lower in adolescents. Lastly, the brain-to-plasma ratio for Δ^9-THC was lower in adolescents than adults (0.26 ± 0.05 *vs.* 1.08 ± 0.2, $p < 0.05$). In sum, the findings suggest that adolescent female mice treated with Δ^9-THC attain lower peak brain concentrations of the drug and its metabolites, relative to adults.

Discussion

Long-term consequences of Δ^9-THC exposure during adolescence have been the object of many animal studies,[13] and the question of whether the pharmacokinetics

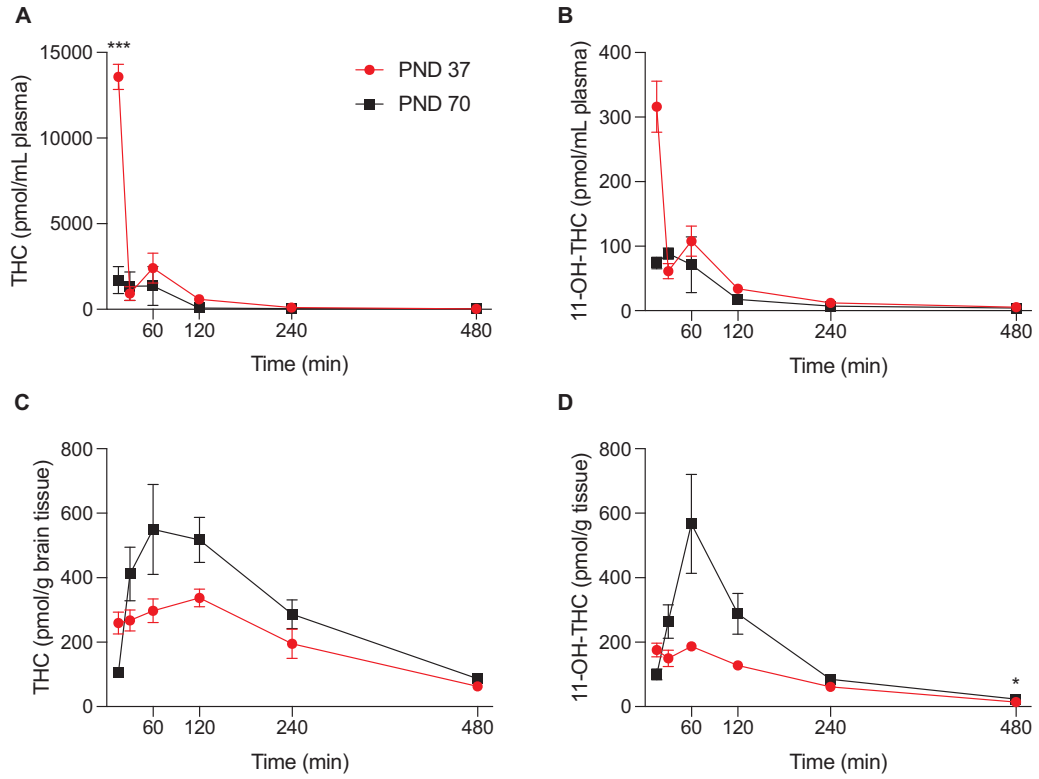

FIG. 1. Plasma (top panels) and brain (bottom panels) concentrations of Δ^9-THC **(A, C)** and 11-OH-THC **(B, D)** in adolescent (PND 37, ●) and adult (PND 70, ■) female C57BL/6 mice. Symbols represent mean ± SEM, $n = 3$ or 4 animals per data point, outlier removed using Grubb's test for outliers,*$p < 0.05$, ***$p < 0.001$, two-way ANOVA. ANOVA, analysis of variance; PND, postnatal day; Δ^9-THC, Δ^9-tetrahydrocannabinol.

of the drug might change in the transition from adolescence to adulthood has been addressed in rats of both sexes as well as in male mice.[5,6] However, the pharmacokinetic properties of Δ^9-THC in female mice remains to be elucidated. Filling this gap is important to understand whether age-dependent changes in the pharmacological response to Δ^9-THC are similar across sex and species. In the present report, we assessed the pharmacokinetics of Δ^9-THC and its two primary metabolites, 11-OH-THC and 11-COOH-THC, after IP administration in adolescent and adult female mice, respectively. The results identify multiple dissimilarities in Δ^9-THC

Table 1. Maximal Concentration (C_{max}) in Plasma, Time at Which Maximal Concentration Was Reached (T_{max}), and Half-Life of Elimination ($t_{1/2}$) for Δ^9-THC and Its Metabolites in Adolescent (PND 37) and Adult (PND 70) Female Mice after IP Injection of 5 mg/kg THC. Data Are Represented as Means of $n = 3$, 4 Animals per Data Point, Outlier Removed Using the Grubb's Test. The Standard Error of the Mean Was Omitted for Clarity

	Adult				Adolescent			
Analyte	C_{max} (pmol/mL)	AUC (pmol•min/mL)	T_{max} (min)	$t_{1/2}$ (min)	C_{max} (pmol/mL)	AUC (pmol•min/mL)	T_{max} (min)	$t_{1/2}$ (min)
Δ^9-THC	1,711	125,230	15	48.1	13,577***	306,887	15	33.9
11-OH-THC	89	14,474	30	—	316*	9,066	15	—
11-COOH-THC	192	57,661	60	—	299	71,802	60	—

*$p < 0.05$.
***$p < 0.001$, Student's t-test.
AUC, area under the curve; Δ^9-THC, Δ^9-tetrahydrocannabinol.

Table 2. Maximal Concentration (C_{max}) in Brain, Time at Which Maximal Concentration Was Reached (T_{max}), and Half-Life of Elimination ($t_{1/2}$) for Δ^9-THC and Its Metabolites in Adolescent (PND 37) and Adult (PND 70) Female Mice after IP Injection of 5 mg/kg THC. Data Are Represented as Means of $n = 3$, 4 Animals per Data Point, Outlier Removed Using the Grubb's Test. The Standard Error of the Mean Was Omitted for Clarity

	Adult				Adolescent			
Analyte	C_{max} (pmol/g)	AUC (pmol•min/g)	T_{max} (min)	$t_{1/2}$ (min)	C_{max} (pmol/g)	AUC (pmol•min/g)	T_{max} (min)	$t_{1/2}$ (min)
Δ^9-THC	550	143,418	60	48.7	337	94,357	120	44.7
11-OH-THC	567	76,232	60	—	187	37,361*	60	—
11-COOH-THC	210	56,568	120	—	145	43,468	120	—

*$p < 0.05$, Student's *t*-test.
AUC, area under the curve; PND, postnatal day; Δ^9-THC, Δ^9-tetrahydrocannabinol.

pharmacokinetics between the two age groups, including potentially relevant differences in peak drug concentrations and brain-to-plasma ratios. These results are relevant to the interpretation of Δ^9-THC exposure studies in adolescent mice.

Previous research has shown that adolescent male mice may be protected from Δ^9-THC entry into the brain,[5] a phenomenon that was not observed in male or female adolescent rats.[5] This raised the question as to whether female mice also bear this protective trait. The present results suggest that they do. In fact, Δ^9-THC plasma AUC was nearly thrice as high in adolescent than adult female mice whereas the brain AUC was 50% lower in the younger group (Tables 1 and 2). Comparatively, the brain-to-plasma ratio was four times lower in adolescent than adult female mice. In our previous study of mouse pharmacokinetics, we found that adolescent males had similar plasma Δ^9-THC AUC, but nearly one-half of the brain AUC and brain-to-plasma ratio of Δ^9-THC compared with adult males.[5] This suggests that female adolescent mice have a similar, if not stronger, level of protection than their male counterparts.[5] Interestingly, this protective phenomenon is not seen in rats of either sex, which suggests that Δ^9-THC pharmacokinetics may be species dependent. We previously attributed the protective mechanism responsible for the higher plasma and lower brain Δ^9-THC concentrations in the male mouse to a less permeable blood–brain barrier, a higher efflux of Δ^9-THC from the brain, faster metabolism, or a weaker diversion of Δ^9-THC to white adipose tissue. Out of these four possible scenarios, only the latter two may be a plausible explanation for differences in mice and rat Δ^9-THC pharmacokinetics since research has shown comparable blood–brain barrier permeability in the two species.[14] Further experimentation is needed to identify potential mechanisms responsible for this apparent species-dependent protection as well as examine the potential impact of the estrous cycle on Δ^9-THC pharmacokinetics.

In sum, our experiments reveal the existence of substantial differences in the absorption and distribution of Δ^9-THC between adolescent and adult female mice—a result with meaningful implications for the interpretation of studies comparing Δ^9-THC effects in mice of these two age groups. In particular, adolescent mice show higher peak concentrations of Δ^9-THC in circulation and reduced entry of the drug into the brain. The mechanisms underpinning these developmental variations deserve further study.

Authors' Contributions

A.T.: Conceptualization, formal analysis, writing—original draft preparation and review and editing. A.M.T.: Conceptualization, investigation, writing—original draft preparation. A.T.: Investigation, formal analysis. F.A.: Methodology. M.H.: Writing—original draft preparation. DP.: Conceptualization, writing—original draft preparation and review and editing, supervision.

Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

Δ^9-THC	Δ^9-tetrahydrocannabinol
11-OH THC	11-hydroxy-Δ^9-THC
11-COOH-THC	11-nor-9-carboxy-Δ9-THC
AUC	area under the curve
CL	clearance
C_{max}	maximal concentration
IP	intraperitoneal
ISTD	internal standards
LC-MS/MS	liquid chromatography/tandem mass spectrometry
PND	post-natal day
$t_{1/2}$	apparent half-life time of elimination
T_{max}	time at which C_{max} was attained
VD	apparent volume of distribution